REMARKS

Reconsideration is requested.

Claims 1-15, 17, 30 and 33 have been canceled, without prejudice.

Claims 16, 18-29, 31, 32 and 34-40 are pending. Claims 35-40 have been added. Claims 35-40 find support in the originally-filed application, including page 15, lines 19-25 relating to sequence length, and the previously examined claims. Specifically, claim 35 combines the details of now-canceled claims 15 and 17; claim 36 combines the details of now canceled claims 15 and claim 28; claim 37 combines the details of now cancelled claims 15 and 17 and claim 28; and claims 38-40 include the details of claim 29. No new matter has been added. The newly added claims are believed to read on the subject matter under examination. Claims 18-27, 31, 32 and 34 have been withdrawn from consideration.

The Examiner's indication that "HBV genotype A specific target sequences consisting of SEQ ID NO: 77, 140 and 193 are free of the prior art" is acknowledged, with appreciation. See page 12 of the Office Action dated September 4, 2008.

A further certified copy of the EP96870053 obtained by the undersigned from the PTO IFW of the copending application no. 10/453,792 (now U.S. Patent No. 7,313,357) is attached. The certified copy is indexed in the IFW of copending application no. 10/453,792 on January 23, 2007. The Examiner previously considered the copending application, as noted by the initialed PTO 1449 Form indexed in the PTO IFW on September 12, 2007 and bearing the OIPE stamp June 14, 2007 (see under U.S. Patent Documents: Document No. US 2004-0029110 A1, and under Other Documents:

File History (FH) of U.S. application Serial No. 10/453,792 retrieved from USPTO IFW June 14, 2007). Application No. 10/453,792 was allowed May 17, 2007. The Examiner therefore is believed to have previously considered the allowed claims of the copending application.

The U.S. Patent No. 7,313,357 is listed on the attached PTO 1449 Form. Return of an initialed copy of the attached PTO 1449 Form, pursuant to MPEP § 609, is requested.

The Examiner is also requested to confirm receipt of the attached certified copy of the priority document. The Examiner is requested to have a BIBDATA SHEET entered in to the PTO IFW of the present application which confirms the applicants claim for priority benefit and receipt of the requirements of 35 USC 119.

The Section 112, first paragraph "written description", rejection of claims 15 and 29 is obviated by the above amendments. The claims have been revised, without prejudice, such that claims 35 and claims dependent therefrom do not include the objected-to recitation of 5 to 50. The specification describes probes of 5 to 50 nucleotides in length, for example, in the paragraph spanning lines 19-25 on page 15, and the paragraph spanning lines 14-25 of page 19; as well as primers of 5-50 nucleotides in length on page 16, lines 21-28. One of ordinary skill in the art will appreciate that the applicants were in possession of the claimed invention at the time the application was filed. Withdrawal of the Section 112, first paragraph "written description", rejection of claims 15 and 29 is requested.

The Section 112, first paragraph "enablement", rejection of claims 15, 16, 17, 28 and 29 is obviated by the above amendments. The claims have been revised, without prejudice, to define probes and primers of the disclosure. One of ordinary skill in the art will be able to make and use the claimed invention, without undue experimentation. Withdrawal of the Section 112, first paragraph "enablement", rejection is requested.

To the extent not obviated by the above amendments, the Section 103 rejections of claims 15, 16 and 29 over Maertens (WO 94/12670) in view of Okamoto (Journal of General Virology 69, 2575-2583, 1988) and Norder (Journal of General Virology 73, 1201-1208, 1992); and of claims "1 and 28"[1] over Maertens, Okamoto, Norder and McDonough (EP 0569237A2), are traversed. Reconsideration and withdrawal of the rejections are requested in view of the above and the following distinguishing comments.

Claim 35 and claims dependent therefrom require the subject matter of now-cancelled claim 17, which the Examiner has found to be patentable over the art of record.

Moreover, the applicants submit that the cited art fails to teach or suggest the primers of claim 28. As described on page 16, line 21 – page 17, line 3, the primers of the claims refer to single stranded oligonucleotide sequences capable of acting as a point of initiation for synthesis of a primer extension product. Therefore, the oligonucleotide as such has been claimed, and not the complementary part thereof which primer will, when applied, amplify a different part of the strain of interest.

McDonough teaches that a complementary part of one of the primers found is suitable to amplify a HBV strain. However it has not been indicated that by using this primer a specific region of the HBV strain viz the HBsAg region could be amplified which region is suitable to genotype HBV strains as for instance HBV genotype A. Other genotypes B – H may be identified by applying the same primers as has been disclosed in the application as filed.

The applicants believe that the methods of Norder et al. involved amplification of the HBsAg region with primers known at that time. Several primers were applied while according to the present patent application, example 1 the primers 134 and 135 may be applied to amplify the HBsAg region of all HBV genotypes and the primers 75 and 76 or 94 may be applied in the second round.

The application of only four (4) primers, for example, offers a very efficient way of amplifying the HBsAg region of many different genotypes which may be genotyped for the A type by hybridization with HBV genotype A specific target sequences in the HBsAg region.

The claims are submitted to be patentable over the cited art of record.

Withdrawal of the Section 103 rejections is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested. The Examiner is requested to contact the undersigned, preferably by telephone, in the event anything further is required in this regard.

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[1] See page 11 of the Office Action dated September 4, 2008. The Examiner is assumed to have rejected claims 15 and 28 over Maertens, Okamoto, Noder and McDonough (EP 0569237A2). Clarification is requested in a new, non-final Office Action in the event the Examiner intended otherwise.

Respectfully submitted,

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certified copy of EP priority document obtained from PTO IFW relating to Appln. No. 10/453,792 IFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

JAN 23 2007

1648

In re Patent Application of		Atty Dkt.	BJS-2551-120
			C# M#
STUYVER, et al.		TC/A.U.	1648
Serial No.	10/453,792	Examiner:	Peng
Filed:	June 4, 2003	Date:	January 23, 2007
Title:	METHOD FOR TYPING AND DETECTING HBV		

Attachments:
(1) Supplemental Submission; and
(2) Certified copy of Priority Document (EP 96870053.4)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

SUPPLEMENTAL SUBMISSION

This is a response/amendment/letter in the above-identified application and includes an attachment which is hereby incorporated by reference and the signature below serves as the signature to the attachment in the absence of any other signature thereon.

☐ **Correspondence Address Indication Form Attached.**

Fees are attached as calculated below:

Total effective claims after amendment **0** minus highest number previously paid for **20** (at least 20) = 0 x $50.00	$0.00 (1202)/$0.00 (2202)	$
Independent claims after amendment **0** minus highest number previously paid for **3** (at least 3) = 0 x $200.00	$0.00 (1201)/$0.00 (2201)	$
If proper multiple dependent claims now added for first time, (ignore improper); add	$360.00 (1203)/$0.00 (2203)	$
Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s)	One Month Extension $120.00 (1251)/$0.00 (2251) Two Month Extensions $450.00 (1252)/$0.00 (2252) Three Month Extensions $1020.00 (1253/$0.00 (2253) Four Month Extensions $1590.00 (1254/$0.00 (2254) Five Month Extensions $2160.00 (1255/$1080.00 (2255)	$
Terminal disclaimer enclosed, add	$130.00 (1814)/ $0.00 (2814)	$

☐ Applicant claims "small entity" status. ☐ Statement filed herewith

Rule 56 Information Disclosure Statement Filing Fee	$180.00 (1806)	$	0.00
Assignment Recording Fee	$40.00 (8021)	$	0.00
Other:		$	**0.00**
	TOTAL FEE ENCLOSED	$	**0.00**

The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Account No. 14-1140. A duplicate copy of this sheet is attached.

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By Atty: B. J. Sadoff, Reg. No. 36,663

Signature: [signature]

1163690

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

STUYVER, et al. Atty. Ref.: 2551-120

Serial No. 10/453,792 Group: 1648

Filed: June 4, 2003 Examiner: Peng

For: METHOD FOR TYPING AND DETECTING HBV

* * * * * * * * * * *

January 23, 2007

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

SUPPLEMENTAL SUBMISSION

Supplemental to the Amendment of January 18, 2007, submitted herewith is a certified copy of the priority document (EP 96870053.4) for the Examiner's convenience.

Respectfully submitted,

NIXON & VANDERHYE P.C.

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Bescheinigung / Certificate / Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. / Patent application No. / Demande de brevet n°

96870053.4

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

M.W. Graham

M.W. GRAHAM

DEN HA[illegible] DE[illegible]
THE HAG[illegible] [illegible]0/06/97
LA HAYE, [illegible]

Europäisches Patentamt | European Patent Office | Office européen des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.: 96870053.4
Demande n°:

Anmeldetag:
Date of filing: 19/04/96
Date de dépôt:

Anmelder:
Applicant(s):
Demandeur(s):
INNOGENETICS N.V.
9052 Gent
BELGIUM

Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Method for typing and detecting HBV

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Internationale Patentklassifikation:
International Patent classification:
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C12Q1/70

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

Method for typing and detecting HBV

The present invention relates to the field of Hepatitis B virus (HBV) diagnosis. More particularly, the present invention relates to the field of HBV genotyping and/or determination of the presence of HBV mutants in test samples.

The present invention relates particularly to a method for the rapid and reliable detection of HBV mutants and/or genotypes occuring in a test sample using specific sets of probes optimized to function together in a reverse-hybridisation assay.

Hepatitis B virus is a small enveloped DNA virus of approximately 3200 bp long. Historically it has been characterized on the basis of immunological reaction of the HBsAg with sets of monoclonal antibodies. Isolates were described as *a,* indicating the common determinant for all different subtypes, followed by subtype-specific combinations: *dw, dr, yw,* or *yr.* The latter are mutually exlusive pairs of determinants, covering the HBsAg amino acids 122 (*d*=lys, *y*=arg) and 160 (*w*=lys, *r*=arg). Several subdeterminats for *w* exist and can be ascribed to the appeareance of certain amino acid variants at codon 127. More recently, a genetic classification has been proposed, based on molecular analysis of the virus. This kind of analysis showed that in total six different genotypes exist, indicated from A to F, with a maximum genetic divergence of 8% when comparing complete genomes (reviewed by Magnius and Norder, 1995).

The genetic variability of HBV might be clinically important. Indeed, the genome variability might include some mechanisms by which HBV avoids immune clearance, and hence induces chronic infection. An important protein marker in inducing immune tolerance, virus elimination, and chronic infection, is HBeAg. The expression of this protein is strictly controled both at the transcriptional and translational level (Li et al., 1993; Okamoto et al., 1990; Yuan et al., 1995; Sato et al., 1995) . Therefore, in the natural course of HBV infection, a well characterized stage of the disease is indicated as HBe-negative

chronic hepatitis B (reviewed by Hadziyannis S.J., 1995). This phase is mostly due to the appeareance of preCore translational stop codon mutations. The overal genetic variability determines the frequency and physical location on the viral genome where these translational stop-codon mutations appear. The transcriptional regulation was proposed to be the mechanism for genotype A (and possibly also F), whereas the translational control was more likely to be found in the other genotypes (Li et al.; 1993; Sato et al., 1995). Contradictory to the translational regulation, it was shown that the transcriptional regulation was unable to block the HBeAg expression completely and was therefore proposed to categorize the phenotype of this mutant as HBe-suppressed, rather than as HBe-negative (Takahashi et al., 1995). In any case, these preCore mutants would lead to a destruction of the pre-existing balance between HBeAg in circulation and the HBc-derived peptides presented by class I HLA molecules on the surface of infected hepatocytes, thereby diminishing the supressive effect of HBeAg on T cells, finally resulting in partial liberation of core-specific CTLs and leading to apoptosis of the infected hepatocytes. In general, after the emergence of the HBe-minus variants, the course of the viral infection is characterized by the progression of chronic hepatitis, which may lead to the development of cirrhosis and hepatocellular carcinoma (Hadziyannis, 1995).

Another issue for which the genetic variability or genotyping of the virus might be of relevance is in the development of vaccines where the response may be mediated by the virus type. Protection against HBV infection of all subtypes is conferred by antibodies to the common 'a' determinant of the HB surface antigen (HBsAg). It has been shown that this 'a' determinant presents a number of epitopes, and that its tertiary structure is most important for its antigenicity. The most important region lies between amino acid 124 and 147, but can be extended from amino acid 114 to 150. An adequate anti-HBs response, built up after vaccination, is in principle fully protective. Infection with a HBV strain harboring mutations in the 'a' determinant region might result in vaçcine failure, because the vaccine-induced humoral immune response does not recognize the mutant HBsAg. The most common vaccine-associated escape mutants are the substitutions of a glycine at position 145 to an arginine

(G145R), K141E, and T126N. But a 2-aa insertion between aa position 122 and 123, and 8-aa insertion between aa 123 and 124 have also been found (Carman et al., 1990, 1995; Crawford, 1990; Waters et al., 1992).

Lamivudine is a (-) enantiomer of 3' thiacytidine, a 2'3'-dideoxynucleoside analogue, and is known to be a potent inhibitor of HBV replication through inhibition of the reverse transcriptase (RT) activity of the HBV polymerase. Lamivudine treatment can result in histological improvements in chronic hepatitis patients, and when given pre- and post-liver transplantation, it can prevent graft reinfection (Honkoop et al., 1995; Naoumov et al., 1995). However, after treatment, a hepatitis flare-up can be observed in most patients, with ALT elevations and HBV DNA that becomes detectable again. This HBV DNA rebound is associated with a new quasi species equilibrium. In a few cases, virus breakthrough during therapy was observed, due to the selection of lamivudine resistent HBV strains. The exact nature of this breakthrough is not yet described, but it is very likely that the accumulation of mutations in the RT part of the Polymerase are responsible for this. A similar mechanism in the HIV RT polymerase has been found, where upon lamivudine treatment, mutations accumulate in the YMDD motif (Gao et al., 1993). This YMDD motif is also present in the RT part of the HBV polymerase, and it is likely to assume that lamivudine selected mutations in HBV will be found in this region.

From all this it can be concluded that the information on the following issues is essential for proper diagnosis, monitoring and follow-up of HBV infections:

- genotype;
- preCore mutations;
- vaccine escape mutations;
- lamivudine selected RT gene mutations.

To obtain all this information using existing technologies is complicated, time-consuming, and requires highly-skilled and experienced personel.

It is thus an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more HBV genotypes possibly present in a biological sample.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more variations in the HBV preS1 region representing one or more HBV genotypes possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of the presence or absence of one or more HBV mutants possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the preCore region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the HBsAg region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for determination of one or more mutations in the polymerase (pol) gene region of HBV possibly present in a biological sample in one single experiment.

More particularly, it is an aim of the present invention to develop a rapid and reliable detection method for the simultaneous determination of one or several HBV genotypes in combination with one or several HBV mutants possibly present in a biological sample in one single experiment.

It is also an aim of the present invention to provide a genotyping assay or method which allows to infer the nucleotide sequence at codons of intrest and/or the HBV mutants of intrest, and/or infer the HBV genotype possibly present in a biological sample.

Even more particularly it is also an aim of the present invention to provide a genotyping assay allowing the detection of the different HBV mutants and genotypes in one single experimental setup.

It is another aim of the present invention to select particular probes able

to discriminate one or more HBV mutations in one of the above mentioned regions of the HBV genome and/or able to discriminate one or more HBV genotypes.

It is more particularly an aim of the present invention to select particular probes able to discriminate wild-type HBV from mutant HBV sequences.

It is also an aim of the present invention to select particular probes able to discriminate wild-type and polymorphic variants of HBV from mutant HBV sequences.

It is also an aim of the present invention to select particular probes able to discriminate HBV genotype sequences.

It is moreover an aim of the present invention to combine a set of selected probes able to genotype HBV and/or discriminate different HBV mutants possibly present in a biological sample, whereby all probes can be used under the same hybridisation and wash conditions.

It is also an aim of the present invention to select primers enabling the amplification of the gene fragment(s) determining the HBV genomic mutations or variations of interest as discussed above.

The present invention also aims at diagnostic kits comprising said probes useful for developing such a genotyping assay and/or assays for detecting monitoring or following-up of HBV infection.

All the aims of the present invention have been met by the following specific embodiments.

As a solution to the above-mentioned problem that it is essential for proper diagnosis, monitoring and follow-up of HBV infection to have information on the genotype of HBV present, the present invention provides an elegant way to tackle problems of such complexity which involves residing to a reverse hybridization approach (particularly on Line Probe Assays strips, as described by Stuyver et al., 1993). Using this technology it is possible to conveniently obtain all essential data in one test run. To achieve this goal, a set of probes needs to be designed and assembled which can detect all relevant polymorphisms in the HBV gene regions of interest.

The present invention thus particularly relates to a method for determining

the presence or absence of one or more HBV genotypes in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV genotype specific target sequence chosen from Figure 1; with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV genotype present in said sample from the differential hybridization signal(s) obtained in step (iv).

The genotype specific target sequences can be any nucleotide variation appearing upon alignment of the different HBV genomes which responsible for classifying a certain HBV isolate as a certain genotype (see Figure 1).

The expression "relevant part of a suitable HBV gene" refers to the part of the HBV gene encompassing the HBV genotype specific target sequence chosen from Figure 1 to be detected.

According to a preferred embodiment of the present invention, step (iii) is performed using a set of at least 2, preferably at least 3, more preferably at least 4 and most preferably at least 5 probes all meticulously designed such that they show the desired hybridization results, when used in a reverse hybridisation assay format, more particularly under the same hybridization and wash conditions implying that each of said probes is able to form a complex upon hybridisation with its target sequence present in the polynucleic acids of the sample as obtained after step (i) or (ii).

The numbering of the HBV gene encoded amino acids and nucleotides is as generally accepted in literature.

More particularly, the present invention relates to a set of at least 2 probes allowing the detection of a genotype specific variation, possibly also including one or more probes allowing the detection of a wild-type sequence, a polymorphic or a mutated sequence at any one of the nucleotide positions showing a sequence diversity upon alignment of all known or yet to be discovered HBV sequences as represented in Figure 1 for all complete HBV genomes found in the EMBL/NCBI/DDBJ/Genbank.

The sets of probes according to the present invention have as a common characteristic that all the probes in said set are designed so that they can be used together in a reverse-hybridization assay, more particularly under similar or identical hybridization and wash conditions as indicated above and below.

Selected sets of probes according to the present invention include probes which allow to differentiate any of the HBV genotype specific nucleotide changes as represented in Figure 1, preferably in the preS1 or HBsAg region of HBV. Said probes being characterized in that they can function in a method as set out above.

In order to solve the above-mentioned problem of obtaining information on the possible presence of HBV mutants in a given sample, the present invention provides an elegant way to tackle this problem which involves residing to a reverse hybridisation approach (particularly on Line Probe Assays strips, as described by Stuyver et al., 1993). Using this technology it is possible to conveniently obtain all essential data in one test run. To achieve this goal, a set of probes needs to be designed and assembled which can detect all relevant mutations and possibly also wild-type sequences or polymorphisms in the HBV gene regions of interest.

Another particularly preferred embodiment of the present invention thus is a method for determining the presence or absence of one or more HBV mutants in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV mutant target sequence chosen from Figure 1, with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U and with said set or probes possibly also comprising one or more wild-type HBV probes corresponding with the respective mutated HBV target sequence;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV mutant(s) present in said sample from the differential hybridization signal(s) obtained in step (iv).

The HBV mutant target sequence according to the present invention can be any sequence including a HBV mutated codon known in the art or yet to be discovered. Particularly preferred HBV mutant target regions are set out below.

In order to solve the problem as referred to above of obtaining information on the essential issues for proper diagnosis of HBV (namely genotype and different mutations particularly in preCore region, vaccine escape mutations and lamivudine selected RT gene mutations), the present invention provides a particularly elegent way to obtain such complex information.

Moreover, careful analysis of the data obtained by the present inventors clearly revealed that combining the information concerning the preCore and escape mutants with data on the genotype is essential to allow adequate interpretation of the results. Hence it is highly advantageous to be able to produce all relevant data simultaneously.

This method for diagnosing HBV mutants, preferably in combination with HBV genotyping, using a set of probes selected as defined above, wherein said set of probes is characterized as being chosen such that for a given HBV mutation disclosed in Figure 1, the following probes are included in said set :

- at least one probe for detecting the presence of the mutated nucleotide(s) at said position;
- at least one probe for detecting the presence of the wild-type nucleotide(s) at said position;
- possibly also (an) additional probe(s) for detecting wild-type polymorphisms at positions surrounding the mutation position.

Inclusion of the latter two types of probes greatly contributes to increasing the sensitivity of said assays as demonstrated in the examples section.

Selected sets of probes according to the present invention include at least one probe, preferably at least two probes, characterizing the presence of a HBV mutation at a nucleotide positions chosen from the preCore region of HBV, more particularly from the following list of codons susceptible to mutations in the HBV preCore region as represented in Figure 1, such as codon 15 in genotype A, and for all genotypes: codon 28, and codon 28 and 29, or in the preCore promoter region.

Said probes being characterized in that they can function in a method as set out above.

An additional embodiment of the present invention includes at least one probe, preferably at least two probes, characterizing the presence of a vaccine escape mutation in a codon positions chosen from the HBsAg region of HBV, more particularly from the list of codons susceptible to mutations in the HBV HBsAg region as in Figure 1, such as at codons 145, 144, 141, 126 or 122.

An additional embodiment of the present invention includes at least one probe, preferably at least two probes, characterizing the presence of a lamivudine resistant mutation in the RT pol gene region of HBV as represented in Figure 1, more particularly in the YMDD motif.

In a selected embodiment said method involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from Figure 1.

According to another selected embodiment said method involves

hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the preCore region as represented in Figure 1.

According to another selected embodiment said method involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence within the HBsAg region as represented in Figure 1.

According to another selected embodiment said method involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the preCore region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence chosen from the HBsAg region as represented in Figure 1.

According to a selected embodiment, said method involves hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence within the preCore region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence chosen from the HBsAg region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a lamivudine resistant HBV mutant target sequence chosen from the RT pol gene region of HBV, as represented in Figure 1.

Particularly preferred embodiments of the invention thus include a set of probes as set out above comprising at least one, preferably at least two, probe(s) for targeting one, preferably two, three or more nucleotide changes appearing in the alignment of HBV genomes as represented in Figure 1.

Even more preferred selected sets of probes according to the present invention include probes derived from two of the same or different regions of HBV bearing HBV mutated nucleotides, or in addition also a third (set of)

probe(s) characterizing the presence of a third HBV mutation at any of the positions shown in Figure 1, or particular combinations thereof.

Particularly preferred is also a set of probes which allows simultaneous detection of HBV mutations at codons 15, 28 and 29 in the PreCore region, possibly in combination with mutations in the preCore promoter regions, in combination with mutations at codons 122, 126, 141, 144, 145 in the HBsAg region, possibly also in combination with mutations in the YMDD motif of the HBV pol gene.

In the instances where the alignment of HBV genomes of Figure 1 is referred to in this invention, it should be construed as referring to an alignment of all existing and future HBV genomes. The existing HBV genome sequences can be deduced from any database, such as the EMBL/NCBI/DDBJ/GENBANK database.

A preferred set of preCore, preS1, HBsAg and RT pol gene probes of this invention are the probes with SEQ ID NO 1 to 107 of Table 1 (see also Figure 1).

A particularly preferred set of probes in this respect is shown in and Figure 2. The probes in Figure 2 were withheld after a first selection for preCore and preS1 probes.

The probes of the invention are designed for attaining optimal performance under the same hybridization conditions so that they can be used in sets of at least 2 probes for simultaneous hybridization. This highly increases the usefulness of these probes and results in a significant gain in time and labour. Evidently, when other hybridization conditions would be preferred, all probes should be adapted accordingly by adding or deleting a number of nucleotides at their extremities. It should be understood that these concomitant adaptations should give rise to essentially the same result, namely that the respective probes still hybridize specifically with the defined target. Such adaptations might also be necessary if the amplified material should be RNA in nature and not DNA as in the case for the NASBA system.

The selection of the preferred probes of the present invention is based on a reverse hybridization assay format using immobilized oligonucleotide probes

present at distinct locations on a solid support. More particularly the selection of preferred probes of the present invention is based on the use of the Line Probe Assay (LiPA) principle which is a reverse hybridization assay using oligonucleotide probes immobilized as parallel lines on a solid support strip (Stuyver et al. 1993; international application WO 94/12670). This approach is particularly advantageous since it is fast and simple to perform. The reverse hybridization format and more particularly the LiPA approach has many practical advantages as compared to other DNA techniques or hybridization formats, especially when the use of a combination of probes is preferable or unavoidable to obtain the relevant information sought.

It is to be understood, however, that any other type of hybridization assay or format using any of the selected probes as described further in the invention, is also covered by the present invention.

The reverse hybridization approach implies that the probes are immobilized to certain locations on a solid support and that the target DNA is labelled in order to enable the detection of the hybrids formed.

The following definitions serve to illustrate the terms and expressions used in the present invention.

The term "HBV mutant" refers to any HBV strain harbouring genomic variations with serological, genetical or clinical consequences.

The term "vaccine escape mutant" is reviewed in the introduction section. The most important region lies between amino acid 124 and 147 of the HBsAg region, but can be extended from amino acid 114 to 150 and in Example 7.

The term "lamivudine resistant mutant" is reviewed in the introduction section and in Example 8.

The term "HBV genotype" refers to HBV strains with an intergenotype variation of 8% or more based on a comparison of complete genomes.

The target material in the samples to be analyzed may either be DNA or RNA, e.g. genomic DNA, messenger RNA, viral RNA or amplified versions thereof. These molecules are also termed polynucleic acids.

It is possible to use genomic DNA or RNA molecules from samples susceptible of containing HBV in the methods according to the present invention.

Well-known extraction and purification procedures are available for the isolation of RNA or DNA from a sample (f.i. in Maniatis et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbour Laboratory Press (1989)).

The term "probe" refers to single stranded sequence-specific oligonucleotides which have a sequence which is complementary to the target sequence to be detected.

The term "target sequence" as referred to in the present invention describes the nucleotide sequence of a part of wild-type, polymorphic or mutant HBV gene sequence to be specifically detected by a probe according to the present invention. This nucleotide sequence may encompass one or several nucleotide changes. Target sequences may generally refer to single nucleotide positions, codon positions, nucleotides encoding amino acid or to sequences spanning any of the foregoing positions. In the present invention said target sequence often includes one, two or more variable nucleotide positions. In the present invention polynucleic acids detected by the probes of the invention will comprise the target sequence against which the probe is detected.

It is to be understood that the complement of said target sequence is also a suitable target sequence in some cases. The target sequences as defined in the present invention provide sequences which should at least be complementary to the central part of the probe which is designed to hybridize specifically to said target region. In most cases the target sequence is completely complementary to the sequence of the probe.

The term "complementary" as used herein means that the sequence of the single stranded probe is exactly the (inverse) complement of the sequence of the single-stranded target, with the target being further defined as the sequence where the mutation to be detected is located.

Since the current application requires the detection of single basepair mismatches, stringent conditions for hybridization are required, allowing in principle only hybridization of exactly complementary sequences. However, variations are possible in the length of the probes (see below). It should also be noted that, since the central part of the probe is essential for its hybridization

characteristics, possible deviations of the probe sequence versus the target sequence may be allowable towards head and tail of the probe when longer probe sequences are used. These variations, which may be conceived from the common knowledge in the art, should however always be evaluated experimentally, in order to check if they result in equivalent hybridization characteristics as the exactly complementary probes.

Preferably, the probes of the invention are about 5 to 50 nucleotides long, more preferably from about 10 to 25 nucleotides. Particularly preferred lengths of probes include 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides. The nucleotides as used in the present invention may be ribonucleotides, deoxyribonucleotides and modified nucleotides such as inosine or nucleotides containing modified groups which do not essentially alter their hybridisation characteristics.

Probe sequences are represented throughout the specification as single stranded DNA oligonucleotides from the 5' to the 3' end. It is obvious to the man skilled in the art that any of the below-specified probes can be used as such, or in their complementary form, or in their RNA form (wherein T is replaced by U).

The probes according to the invention can be prepared by cloning of recombinant plasmids containing inserts including the corresponding nucleotide sequences, if need be by cleaving the latter out from the cloned plasmids upon using the adequate nucleases and recovering them, e.g. by fractionation according to molecular weight. The probes according to the present invention can also be synthesized chemically, for instance by the conventional phosphotriester method.

The term "solid support" can refer to any substrate to which an oligonucleotide probe can be coupled, provided that it retains its hybridization characteristics and provided that the background level of hybridization remains low. Usually the solid substrate will be a microtiter plate, a membrane (e.g. nylon or nitrocellulose) or a microsphere (bead) or a chip. Prior to application to the membrane or fixation it may be convenient to modify the nucleic acid probe in order to facilitate fixation or improve the hybridization efficiency. Such

modifications may encompass homopolymer tailing, coupling with different reactive groups such as aliphatic groups, NH_2 groups, SH groups, carboxylic groups, or coupling with biotin, haptens or proteins.

The term "labelled" refers to the use of labelled nucleic acids. Labelling may be carried out by the use of labelled nucleotides incorporated during the polymerase step of the amplification such as illustrated by Saiki et al. (1988) or Bej et al. (1990) or labelled primers, or by any other method known to the person skilled in the art. The nature of the label may be isotopic (^{32}P, ^{35}S, etc.) or non-isotopic (biotin, digoxigenin, etc.).

The term "primer" refers to a single stranded oligonucleotide sequence capable of acting as a point of initiation for synthesis of a primer extension product which is complementary to the nucleic acid strand to be copied. The length and the sequence of the primer must be such that they allow to prime the synthesis of the extension products. Preferably the primer is about 5-50 nucleotides long. Specific length and sequence will depend on the complexity of the required DNA or RNA targets, as well as on the conditions of primer use such as temperature and ionic strength.

The expression "suitable primer pair" in this invention refers to a pair of primers allowing the amplification of part or all of the HBV gene for which probes are immobilized.

The fact that amplification primers do not have to match exactly with the corresponding template sequence to warrant proper amplification is amply documented in the literature (Kwok et al., 1990).

The amplification method used can be either polymerase chain reaction (PCR; Saiki et al., 1988), ligase chain reaction (LCR; Landgren et al., 1988; Wu & Wallace, 1989; Barany, 1991), nucleic acid sequence-based amplification (NASBA; Guatelli et al., 1990; Compton, 1991), transcription-based amplification system (TAS; Kwoh et al., 1989), strand displacement amplification (SDA; Duck, 1990; Walker et al., 1992) or amplification by means of Qß replicase (Lizardi et al., 1988; Lomeli et al., 1989) or any other suitable method to amplify nucleic acid molecules known in the art.

The oligonucleotides used as primers or probes may also comprise

nucleotide analogues such as phosphorothiates (Matsukura et al., 1987), alkylphosphorothiates (Miller et al., 1979) or peptide nucleic acids (Nielsen et al., 1991; Nielsen et al., 1993) or may contain intercalating agents (Asseline et al., 1984).

As most other variations or modifications introduced into the original DNA sequences of the invention these variations will necessitate adaptions with respect to the conditions under which the oligonucleotide should be used to obtain the required specificity and sensitivity. However the eventual results of hybridisation will be essentially the same as those obtained with the unmodified oligonucleotides.

The introduction of these modifications may be advantageous in order to positively influence characteristics such as hybridization kinetics, reversibility of the hybrid-formation, biological stability of the oligonucleotide molecules, etc.

The "sample" may be any biological material taken either directly from the infected human being (or animal), or after culturing (enrichment). Biological material may be e.g. expectorations of any kind, broncheolavages, blood, skin tissue, biopsies, sperm, lymphocyte blood culture material, colonies, liquid cultures, faecal samples, urine etc.

The sets of probes of the present invention will include at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more probes. Said probes may be applied in two or more (possibly as many as there are probes) distinct and known positions on a solid substrate. Often it is preferable to apply two or more probes together in one and the same position of said solid support.

For designing probes with desired characteristics, the following useful guidelines known to the person skilled in the art can be applied.

Because the extent and specificity of hybridization reactions such as those described herein are affected by a number of factors, manipulation of one or more of those factors will determine the exact sensitivity and specificity of a particular probe, whether perfectly complementary to its target or not. The importance and effect of various assay conditions, explained further herein, are known to those skilled in the art.

The stability of the [probe : target] nucleic acid hybrid should be chosen to be compatible with the assay conditions. This may be accomplished by avoiding long AT-rich sequences, by terminating the hybrids with G:C base pairs, and by designing the probe with an appropriate Tm. The beginning and end points of the probe should be chosen so that the length and %GC result in a Tm about 2-10°C higher than the temperature at which the final assay will be performed. The base composition of the probe is significant because G-C base pairs exhibit greater thermal stability as compared to A-T base pairs due to additional hydrogen bonding. Thus, hybridization involving complementary nucleic acids of higher G-C content will be stable at higher temperatures.

Conditions such as ionic strength and incubation temperature under which a probe will be used should also be taken into account when designing a probe. It is known that hybridization will increase as the ionic strength of the reaction mixture increases, and that the thermal stability of the hybrids will increase with increasing ionic strength. On the other hand, chemical reagents, such as formamide, urea, DMSO and alcohols, which disrupt hydrogen bonds, will increase the stringency of hybridization. Destabilization of the hydrogen bonds by such reagents can greatly reduce the Tm. In general, optimal hybridization for synthetic oligonucleotide probes of about 10-50 bases in length occurs approximately 5°C below the melting temperature for a given duplex. Incubation at temperatures below the optimum may allow mismatched base sequences to hybridize and can therefore result in reduced specificity.

It is desirable to have probes which hybridize only under conditions of high stringency. Under high stringency conditions only highly complementary nucleic acid hybrids will form; hybrids without a sufficient degree of complementarity will not form. Accordingly, the stringency of the assay conditions determines the amount of complementarity needed between two nucleic acid strands forming a hybrid. The degree of stringency is chosen such as to maximize the difference in stability between the hybrid formed with the target and the nontarget nucleic acid. In the present case, single base pair changes need to be detected, which requires conditions of very high stringency.

The length of the target nucleic acid sequence and, accordingly, the

length of the probe sequence can also be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly complementary base sequence will normally primarily determine hybrid stability. While oligonucleotide probes of different lengths and base composition may be used, preferred oligonucleotide probes of this invention are between about 5 to 50 (more particularly 10-25) bases in length and have a sufficient stretch in the sequence which is perfectly complementary to the target nucleic acid sequence.

Regions in the target DNA or RNA which are known to form strong internal structures inhibitory to hybridization are less preferred. Likewise, probes with extensive self-complementarity should be avoided. As explained above, hybridization is the association of two single strands of complementary nucleic acids to form a hydrogen bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in a hybrid that it will be less able to participate in formation of a new hybrid. There can be intramolecular and intermolecular hybrids formed within the molecules of one type of probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. By designing a probe so that a substantial portion of the sequence of interest is single stranded, the rate and extent of hybridization may be greatly increased. Computer programs are available to search for this type of interaction. However, in certain instances, it may not be possible to avoid this type of interaction.

Standard hybridization and wash conditions are disclosed in the Materials & Methods section of the Examples. Other conditions are for instance 3X SSC (Sodium Saline Citrate), 20% deionized FA (Formamide) at 50°C.

Other solutions (SSPE (Sodium saline phosphate EDTA), TMACl (Tetramethyl ammonium Chloride), etc.) and temperatures can also be used provided that the specificity and sensitivity of the probes is maintained. If need be, slight modifications of the probes in length or in sequence have to be carried

out to maintain the specificity and sensitivity required under the given circumstances.

In a more preferential embodiment, the above-mentioned polynucleic acids from step (i) or (ii) are hybridized with at least two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more of the above-mentioned target region specific probes, preferably with 5 or 6 probes, which, taken together, cover the "mutation region" of the relevant HBV gene.

The term "mutation region" means the region in the relevant HBV gene sequence where at least one mutation encoding a HBV mutant is located in a preferred part of this mutation region is represented in figure 1.

Apart from mutation regions as defined above the HBV wild-type or mutant genomes may also show polymorphic nucleotide variations at positions other than those referred to as genotype specific or mutant specific variated positions as shown in Figure 1.

Since some mutations may be more frequently occurring than others, e.g. in certain geographic areas or in specific circumstances (e.g. rather closed communities) it may be appropriate to screen only for specific mutations, using a selected set of probes as indicated above. This would result in a more simple test, which would cover the needs under certain circumstances.

In order to detect HBV genotypes and/or HBV mutants with the selected set of oligonucleotide probes, any hybridization method known in the art can be used (conventional dot-blot, Southern blot, sandwich, etc.).

However, in order to obtain fast and easy results if a multitude of probes are involved, a reverse hybridization format may be most convenient.

In a preferred embodiment the selected set of probes are immobilized to a solid support in known distinct locations (dots, lines or other figures). In another preferred embodiment the selected set of probes are immobilized to a membrane strip in a line fashion. Said probes may be immobilized individually or as mixtures to delineated locations on the solid support.

A specific and very user-friendly embodiment of the above-mentioned preferential method is the LiPA method, where the above-mentioned set of

probes is immobilized in parallel lines on a membrane, as further described in the examples.

The invention also provides for a set of primers allowing amplification of the region of the respective HBV gene to be detected by means of probes. Examples of such primers of the invention are given in Table 1 and Figure 1.

Primers may be labelled with a label of choice (e.g. biotine). Different primer-based target amplification systems may be used, and preferably PCR-amplification, as set out in the examples. Single-round or nested PCR may be used.

The invention also provides a kit for diagnosing or monitoring HBV genotypes and/or HBV mutants present in a biological sample comprising the following components:

(i) when appropriate, a means for releasing, isolating or concentrating the polynucleic acids present in said sample;

(ii) when appropriate, at least one suitable primer pair;

(iii) at least two of the probes as defined above, possibly fixed to a solid support;

(iv) a hybridization buffer, or components necessary for producing said buffer;

(v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

(vii) when appropriate, a means for attaching said probe to a known location on solid support.

The term "hybridization buffer" means a buffer enabling a hybridization reaction to occur between the probes and the polynucleic acids present in the sample, or the amplified products, under the appropriate stringency conditions.

The term "wash solution" means a solution enabling washing of the hybrids formed under the appropriate stringency conditions.

As illustrated in the Examples section, a line probe assay (LiPA) was designed for screening for HBV genotypes and/or HBV mutants. The principle of the assay is based on reverse hybridization of an amplified polynucleic acid fragment such as a biotinylated PCR fragment of the HBV gene onto short

oligonucleotides. The latter hybrid can then, via a biotine-streptavidine coupling, be detected with a non-radioactive colour developing system.

The following examples only serve to illustrate the present invention. These examples are in no way intended to limit the scope of the present invention.

FIGURE AND TABLE LEGENDS

Figure 1. Alignment of 35 complete HBV genomes. Isolates belonging to genotype A are: HBVXCPS, HBVADW, HVHEPB, S50225, HPBADWZCG; genotype B: HPBADW3, HPBADWZ, HPBADW1, HPBADW2; genotype C: HPBCGADR, HBVADRM, HPBADRA, HPBCG, HEHBVAYR, HBVADR, HBVADR4, HPBADR1C, HPBADRC, HBVPREX, HPBETNC, HHVBC, HHVCCHA; genotype D: HBVAYWMCG, HBVAYWC, HBVAYWCI, HBVAYWE, HBVDNA, HPBHBVAA, XXHEPAV, HBVORFS; genotype E: HHVBE4, HHVBBAS; and genotype F: HHBF, HHVBFFOU, HBVADW4A. To preserve alignment, several gaps were created in the alignment and are indicated with /. Positions of start and end of the different HBV encoded genes is indicated: HBsAg: hepatitis B surface antigen (small surface antigen); HBx: hepatitis B X protein; HB Pol: hepatits B polymerase protein, encoding a terminal protein, a spacer, a RT/DNA polymerase region, and an RNAse H activity; HBcAg: hepatitis B Core antigen; HBpreS1Ag: hepatitis B preS1 antigen (large surface antigen); HBpreS2Ag: hepatitis B preS2 antigen (middle surface antigen). The position of the PCR primers is indicated with a large box over all 35 sequences. The polarity of the PCR primer can be deduced from the position of the name above these boxes: left = antisense primer; right = sense primer. LiPA probes are indicated with small boxes, the number of the probe is indicated on left of the alignment, and correspond met the probe positions in Figure 2.

Figure 2: LiPA HBV design. The content of HBV LiPA strip is detailed. For each line number, the region on the viral genome is indicated, together with the genotype that is detected, the probe number that corresponds with the boxes from the alignement in Figure 1, and the sequence of the probe.

Figure 3: Combined result of genotype determination and preCore scanning on 24 samples. The interpretation of each sample is given under each strip. Probe reactivities on lines 3 to 14 are obtained from the preS1 PCR fragment, probe

reactivities on lines 15 to 27 are due to the preCore PCR fragment. Genotypes are indicated from A to F. The interpretation for the preCore region is as follows: W = wild type; M = mutant; I = indeterminate, meaning that no reactivity is observed, which is due to mutations that could not yet be detected with the selected probes; mix = mixture of wild type and mutant; interpretation of codon 15 is only relevant for genotype A, the absence of reactivity on HBPr 45 for genotypes B to F is of no use as is indicated with - (not applicable). Since the presence or absence of preCore mutations has effect on the serological HBeAg status, this is also indicated.

Table 1: Overview of all primers and probes referred to in the Figures with an indication of their respective SEQ ID NO. Primers from the PreS1 region include 1, 106, 2 (sense primers) and 4, 107 and 3 (antisense primers). Primers from the HBsAg region include 75 and 104 (sense primers) and 76, 94 and 105 (antisense primers). Primers from the PreCore region include 69, 86, 70, 87, 6 and 84 (sense primers) and 7, 85 and 8 (antisense primers). The remaining nucleotides are probes from the PreCore, PreS1, HBsAg and pol RT gene regions of HBV as indicated in Figure 1.

Table 1 : HBV probe and primer design

Name	Sequence	SEQ ID NO
HBPr1	GGGTCACCATATTCTTGGG	1
HBPr2	GAACAAGAGCTACAGCATGGG	2
HBPr3	CCACTGCATGGCCTGAGGATG	3
HBPr4	GTTCCT/GGAACTGGAGCCACCAG	4
HBPr5	TCTTTGTATTAGGAGGCTGTAG	5
HBPr6	GCTGTAGGCATAAATTGGTCTG	6
HBPr7	CTCCACAGT/AAGCTCCAAATTC	7
HBPr8	GAAGGAAAGAAGTCAGAAGGC	8
HBPr9	TGGCTTTGGGGCATGG	9
HBPr10	TGGCTTTAGGGCATGG	10
HBPr11	TGGCTTTAGGACATGG	11
HBPr12	AAGTTGCATGGTGCTG	12
HBPr13	CACCTCTGCCTAATCAT	13
HBPr14	TGGGGTGGAGCCCTCAG	14
HBPr15	GCCAGCAGCCAACCAG	15
HBPr16	CCCATGGGGGACTGT	16
HBPr17	AACCCCAACAAGGATG	17
HBPr18	TCCACCAGCAATCCT	18
HBPr19	TGGGGGAAGAATATTT	19
HBPr20	AAATTCCAGCAGTCCC	20
HBPr21	GTTCCCAACCCTCTGG	21
HBPr22	AACCTCGCAAAGGCAT	22
HBPr23	TGCATTCAAAGCCAAC	23
HBPr24	TACTCACAACTGTGCC	24
HBPr25	ACCCTGCGTTCGGAGC	25
HBPr26	CAGGAAGACAGCCTAC	26
HBPr27	GATCCAGCCTTCAGAG	27
HBPr28	ATGCTCCAGCTCCTAC	28
HBPr29	GCTTTCTTGGACGGTC	29
HBPr30	CTACCCCAATCACTCC	30
HBPr31	AGCACCTCTCTCAACG	31
HBPr32	CCAATGGCAAACAAGG	32
HBPr33	CTGAGGGCTCCACCCCA	33
HBPr34	ATGCAACTTTTTCACC	34
HBPr35	ATCTCTTGTACATGTC	35
HBPr36	ATCTCATGTTCATGTC	36
HBPr37	CAGTGGGACATGTACA	37
HBPr38	CAGTAGGACATGAACA	38
HBPr39	CTGTTCAAGCCTCCAA	39
HBPr40	AGCCTCCAAGCTGTGC	40
HBPr41	AAAGCCACCCAAGGCA	41
HBPr42	TGGCTTTAGGACATGGA	42
HBPr43	GACATGTACAAGAGATGA	43
HBPr44	GACATGAACATGAGATGA	44
HBPr45	TGTACATGTCCCACTGTT	45
HBPr46	TGTTCATGTCCTACTGTT	46
HBPr47	ACTGTTCAAGCCTCCAAG	47
HBPr48	GGCACAGGCTTGGAGGCTT	48
HBPr49	AAAGCCACCCAAGGCACA	49
HBPr50	CCCAGAGGGTTGGGAAC	50
HBPr51	CAGCATGGGGCAGAATCT	51
HBPr52	TCCACCAGCAATCCTCTG	52
HBPr53	GGATCCAGCCTTCAGAGC	53
HBPr54	TCAGGAAGACAGCCTAC	54
HBPr55	TTCAACCCCAACAAGGATC	55

HBPr56	AATGCTCCAGCTCCTAC	56
HBPr57	CTGCATTCAAAGCCAACT	57
HBPr58	CCCCATGGGGGACTGTTG	58
HBPr59	CATACTCACAACTGTGCCA	59
HBPr60	GGGCTTTCTTGGACGGTCC	60
HBPr61	CTCTCGAATGGGGGAAGA	61
HBPr62	CCTACCCCAATCACTCCA	62
HBPr63	AGCACCTCTCTCAACGACA	63
HBPr64	GCAAATTCCAGCAGTCCCG	64
HBPr65	GCCAATGGCAAACAAGGTA	65
HBPr66	GACATGAACATGAGATG	66
HBPr67	GGACATGAACAAGAGAT	67
HBPr68	GACATGTACAAGAGATG	68
HBPr69	ACATAAGAGGACTCTTGGAC	69
HBPr70	TACTTCAAAGACTGTGTGTTTA	70
HBPr71	ACAAAGACCTTTAAC/TCT	71
HBPr72	ACAAAGATCATTAAC/TCT	72
HBPr73	TTCCACCAGCAATCCTC	73
HBPr74	GATCCAGCCTTCAGAGC	74
HBPr75	CAAGGTATGTTGCCCGTTTGTCC	75
HBPr76	CCAAACAGTGGGGGAAAGCCC	76
HBPr77	CTACGGATGGAAATTGC	77
HBPr78	TACGGACGGAAACTGC	78
HBPr79	TTCGGACGGAAACTGC	79
HBPr80	CTTCGGACGGAAATTGC	80
HBPr81	CTACGGATAGAAATTGC	81
HBPr82	CTTCGGACAGAAATTGC	82
HBPr83	CTATGGGAGTGGGCCTCAGT/CC	83
HBPr84	GCTGTAGGCATAAATTGGTCTG	84
HBPr85	CTCCACAGT/AAGCTCCAAATTC	85
HBPr86	ACATAAGAGGACTCTTGGAC	86
HBPr87	TACTTCAAAGACTGTGTGTTTA	87
HBPr88	TAGGTTAAAGGTCTTTGT	88
HBPr89	TAGGTTAATGATCTTTGT	89
HBPr90	CATGTCCCACTGTTCAA	90
HBPr91	CATGTCCTACTGTTCAA	91
HBPr92	TTCTGCCCCATGCTGTA	92
HBPr93	TTCTGCCCCATGCTGTAG	93
HBPr94	GGTAA/TAAAGGGACTCAC/AGATG	94
HBPr95	TCAGCTATATGGATGAT	95
HBPr96	CAGCTATATGGATGAT	96
HBPr97	TTCAGCTATATGGATG	97
HBPr98	TCAGTTATATGGATGAT	98
HBPr99	TTTCAGTTATATGGATG	99
HBPr100	TTTAGTTATATGGATGA	100
HBPr101	TCAGCTATGTGGATGAT	101
HBPr102	TCAGTTATGTGGATGAT	102
HBPr103	TTTCAGCTATGTGGATG	103
HBPr104	CAAGGTATGTTGCCCGTTTGTCC	104
HBPr105	GGT/CAA/TAAAGGGACTCAC/AGAT	105
HBPr106	GGGTCACCATATTCTTGGG	106
HBPr107	GTTCCT/GGAACTGGAGCCACCAG	107

EXAMPLES

Example 1. *HBV DNA preparation and PCR amplification*

Serum samples were collected from HBsAg-positive individuals and stored at minus 20°C until use in 0.5 ml aliquots. To prepare the viral genome, 18 μl serum was mixed with 2 μl 1N NaOH and incubated at 37°C for 60 minutes. The denaturation was stopped and neutralized by adding 20 μl of 0.1N HCl. After a 15 minutes centrifugation step, the supernatant was collected and the pellet discarded. PCR was carried out on this lysate as follows: 32 μl H_2O was mixed with 5 μl of 10x PCR buffer, 1 μl 10 mM dXTPs, 1 μl of each biotinylated primer (10 pmol/μl), 10 μl of serum lysate, and 2 U Taq enzyme. The amplification scheme contained 40 cycles of 95°C 1 min, annealing at 45°C for 1 min, and extension at 72°C for 1 min. Amplification products were visualized on 3% agarose gel.

The outer primer set for preS1 has the following sequence:

outer sense: HBPr 1: 5'-bio-GGGTCACCATATTCTTGGG- 3'

outer antisense HBPr 4: 5'-bio-GTTCC(T/G)GAACTGGAGCCACCAG-3'

The outer primer set for preCore has the following sequence:

outer sense: HBPr 69: 5'-bio-ACATAAGAGGACTCTTGGAC-3'

outer antisense: HBPr 8: 5'-bio-GAAGGAAAGAAGTCAGAAGGC-3'

Samples that were negative in the first round PCR were retested in a nested reaction composed of the following: μl H_2O, 5 μl 10x Taq buffer, 1 μl 10 mM dXTPs, 1 μl of each nested primer (10 pmol/μl), 1 μl of the first round PCR product, and 2 U Taq polymerase. The amplification scheme was identical as for the first round PCR. The sequence of the nested primers were as follows, for the pre S1 region:

nested sense HBPr 2: 5'-bio-GAACAAGAGCTACAGCATGGG- 3'

nested antisense HBPr 3: 5'-bio-CCACTGCATGGCCTGAGGATG-3';

and for the preCore region:

nested sense HBPr 70: 5'-bio-TACTTCAAAGACTGTGTGTTTA-3'

nested antisense HBPr 7: 5'-bio- CTCCACAG(T/A)AGCTCCAAATTC-3'

In a second reaction the HBsAg region can be amplified in a similar protocol by using the following primers: HBPr 75: 5'-bio-CAAGGTATGTTGCCCGTTTGTCC-3' in combination with either HBPr 76: 5'-bio-CCAAACAGTGGGGGAAAGCCC-3'; or with HBPr 94: 5'-bio-GGTA(A/T)AAAGGGACTCA(C/A)GATG-3'.

Example 2. *Preparation of the Line Probe Assays*

Probes were designed to cover the universal, genotypic and mutant motifs. In principle only probes that discriminate between one single nucleotide variation were retained. However, for certain polymorphisms at the extreme ends of the probe, cross-reactivity was tolerated. Specificity was reached experimentally for each probe individually after considering the % (G+C), the probe length, the final concentration, and hybridization temperature. Optimized probes were provided enzymatically with a poly-T-tail using the TdT (Pharmacia) in a standard reaction condition. Briefly, 400 pmol probe was incubated at 37°C in a 30 μl reaction mix containing 5.3 mM dTTP, 25 mM Tris.HCL pH 7.5, 0.1 M sodium cacodylate, 1 mM $CoCl_2$, 0.1 M DTT and 170 U terminal deoxynucleotidyl transferase (Pharmacia). After one hour incubation, the reaction was stopped and the tailed probes were precipitated and washed with ice-cold ethanol. Probes were dissolved in 6x SSC at their respectively specific concentrations and applied as horizontal lines on membrane strips in concentrations between 0.2 and 2.5 pM/ml. Biotinylated DNA was applied alongside as positive control (LiPA line 1) . The oligonucleotides were fixed to the membrane by baking at 80°C for 12 hours. The membrane was than sliced into 4 mm strips. The design of this strip is indicated in Figure 2.

Example 3. *LiPA test performance*

Equal volumes (10 μl each) of the biotinylated PCR fragment and of the denaturation solution (DS; 400 mM NaOH/10 mM EDTA) were mixed in test troughs and incubated at room temperature for 5 minutes. Then, 2 ml of the 37°C prewarmed hybridization solution (HS, 3x SSC/0.1% SDS) was added,

followed by the addition of one strip per test trough. Hybridisation occured for 1 hour at 50 ± 0.5°C in a closed shaking water bath. The strips were washed twice with 2 ml of stringent wash solution (3x SSC/0.1% SDS) at room temperature for 20 seconds, and once at 50°C for 30 minutes. Following this stringent wash, strips were rinsed two times with 2 ml of the Innogenetics standard Rinse Solution (RS). Strips were incubated on a rotating platform with the alkaline phosphatase-labelled streptavidin conjugate, diluted in standard Conjugate Solution for 30 minutes at room temperature (20 to 25°C). Strips were than washed twice with 2 ml of RS and once with standard Substrate Buffer (SB), and the colour reaction was started by adding BCIP and NBT to the SB. After maximum 30 minutes at room temperature, the colour reaction was stopped by replacing the colour compounds by distilled water. Immediately after drying, the strips were interpreted. Reactivities were considered positive whenever the reactivity was stronger than the reaction on the negative control. Strips can be stored on a dry dark place. The complete procedure described above can also be replaced by the standardized Inno-LiPA automation device (*auto*-LiPA).

Example 4. *Selection of reference material.*

Selected preCore PCR products, amplified without 5' biotine primers, are cloned into the pretreated EcoRV site of the pGEMT vector (Promega). Recombinant clones are selected after α-complementation and restriction fragment length analysis, and sequenced with plasmid primers. Other biotinylated fragments were directly sequenced with a dye-terminator protocol (Applied Biosystems) using the amplification primers. Alternatively, nested PCR was carried out with analogs of the HBPr70 and HBPr7 primers, in which the biotine group was replaced with the T7- and SP6-primer sequence, respectively. These amplicons were than sequenced with an SP6- and T7-dye-primer procedure. By doing so, a cloned reference panel of the 6 different HBV genotypes is prepared which is necessary for optimizing LiPA probes. Likewise, different sequence patterns in the preCore promoter region and in the preCore open reading frame are cloned and sequenced and are used for

optimizing the preCore probes.

Example 5: *Genotyping HBV-infected serum samples.*

Only after creating a sequence alignment as shown in Figure 1, it became clear which regions could be useful for HBV genotyping. The preS1 region seems to be most suitable because of the high degree of variability. Probes were therefore designed to cover most of these variable regions as shown in Table 1. Only a limited selection of probes was retained because of their specific reaction with the reference panel. The most important ones are indicated as boxed regions in Figure 1 and are also indicated in Table 1. These selected probes were than applied in a LiPA format indicated in Figure 2, as line number 2 to 14. Some of the probes could be applied together in one line, because of their universal character, while others need to applied seperately. With the selection of probes thus obtained, serum samples collected at different parts of the world (Europe, South-America, Africa, Middle-East) were tested. The upper part of figure 3 shows the reactivity of a selection of samples on these probes. Genotyping of these samples is straightforward, with samples 2 to 8 belonging to genotype A, samples 9 and 10 belonging to genotype B, samples 11 and 12 belonging to genotype C, samples 13 to 19 belonging to genotype D, samples 20 to 23 belonging to genotype E, and sample 24 belonging to genotype F.

Example 6. *Scanning the preCore region for mutations.*

HBeAg expression can be regulated at the transcriptional and translational level. It is postulated that a transcriptional regulation exists due to the presence of a dinucleotide variation in the promoter region of the preCore mRNA. Probes covering the wild type (e.g. probe HBPr 88) and the mutant (e.g. HBPr 89) motif were selected and their positions are indicated in the alignment shown in Figure 1, and applied on the LiPA strip as line 15 and 16 (Figure 2).
At the translational level, much more mutations might arise, all possibly resulting in abrogation of the HBeAg expression: any mutations at codon 1 (ATG) destroying translation initiation, codon 2 (CAA to TAA), codon 7 (TGC to TGA), codon 12 (TGT to TGA), codon 13 in genotype B, C, D, E, F (TCA to TGA or

TAA), codon 14 (TGT to TGA), codon 18 (CAA to TAA), codon 21 (AAG to TAG), codon 23 (TGC to TGA), codon 26 (TGG to TAG or TGA), codon 28 (TGG to TAG or TGA). However, due to secondary contrain of the encapsidation signal, most of the mutations occur at codon 28 (TGG to TAG). Along with the mutation at codon 28, a second mutation at codon 29 (GGC to GAC) is often observed. In the case of genotype A and again as a consequence of the secondary constrain, stop codon mutations at codon 28 are only likely to occur after selection of a codon 15 mutation (CCC to CCT). Hence, correct interpretation of preCore mutations is genotype dependent. In addition to the above mentioned stop codons, a huge amount of different deletion- or insertion-mutations in the preCore open reading frame might give essentially the same result.

In order to develop a sensitive assay to detect the relevant mutations and the hypothetical mutations, a probe scanning procedure was developed. Partially overlapping probes were designed and applied in a LiPA format (Figure 2, line 17 to 27). In this assay format, wild type sequences over the complete preCore region, together with the codon 15 variation for genotype A versus non-A genotypes, and the most common mutations at codon 28 (TAG) and the combination of codon 28 and 29 (TAGGAC) are positively recognized. Absence of reactivity at one of the other probes is always indicative for the presence of a variation. The exact nature of this variation can then be revealed by sequence analysis or with further designed LiPA probes.

Figure 3 shows the reactivity of the selected genotyped samples on the probes for the preCore region. Samples were previously tested for the presence of HBeAg or for anti-HBe. The interpretation of the reactivity on the LiPA probes for each sample is indicated below each strip. This approach allowed for the simultaneous screening of a sample for preCore mutations and the characterization of the viral genotype.

Example 7. Detection of mutants in the HBsAg region.

Vaccine escape mutants have been described. The most commonly found mutant is the variation at codon 145 of HBsAg (G145R or GGA to AGA). LiPA

probes are designed to detect wild type and mutant probes. Genotypic variations are present in the vicinity of codon 145. Therefore, genotype A is covered by probe 77, genotype B by probe 78, genotype C by probe 79, and genotype D/E by probe 80. Hence, in principle, it is possible to genotype and detect the wild type strains of the virus in one single experiment. Mutant probes are covered by probe 84 and 82 for genotype A and D, respectively. Probe 83 can be used as a positive control in these experiments. Further detection of mutants in the *a* determinant region is possible by means of a probe scanning approach. Herefore, probes are designed to cover the wild type sequence of the different genotypes over the HBsAg epitope region and applied in a LiPA format. Again here, absence of staining at one of these probes is indicative for the presence of a mutant strain. The exact nature of this variant is then determined by sequencing analysis.

Example 8. *Detection of HBV strains resistant to lamivudine.*

Through analogy with HIV and the resistance against the anti-viral compound 3TC (lamivudine or (-)-ß-1-2',3'-dideoxy-3'-thiacytidine), it is predicted that upon treatment of HBV-infected patients with 3TC, viral strains will be selected showing resistance at the YMDD motif in the HB pol gene. The YMDD motif is physically located in the HBsAg region, but is encoded in another reading frame. Hence, this part of the HBV pol region is amplified with the primer combination HBPr 74-HBr 94, but not with the combination HBPr 74-HBr 76. Probes covering the wild type YMDD motif and YVDD mutant motif are indicated in Figure 1, respectively probes 95 to 100 and 101 to 103. With this assay, resistant HBV strains can be easily detected. The combined detection of the YMDD motif and preCore mutants might be clinically important in prediction and prognosis of further treatment.

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4

CLAIMS

1. Method for determining the presence or absence of one or more HBV genotypes in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV genotype specific target sequence chosen from Figure 1, with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV genotype present in said sample from the differential hybridization signal(s) obtained in step (iv).

2. Method for determining the presence or absence of one or more HBV mutants in a biological sample, comprising:

(i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample;

(ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;

(iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two nucleotide probes hybridizing specifically to a HBV mutant target sequence chosen from Figure 1, with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the

polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T of said target sequence is replaced by U and with said set or probes possibly also comprising one or more wild-type HBV probes corresponding with the respective mutated HBV target sequence;

(iv) detecting the hybrids formed in step (iii);

(v) inferring the HBV mutant(s) present in said sample from the differential hybridization signal(s) obtained in step (iv).

3. Method according to claim 2, wherein step (iii) consists of hybridizing with at least two nucleotide probes hybridizing specifically to a HBV mutant target sequence chosen from the preCore region of HBV as represented in Figure 1.

4. Method according to claim 2 for detecting the presence or absence of one or more HBV vaccine escape mutants in said sample, wherein step (iii) consists of hybridizing with at least two nucleotide probes hybridizing specifically to a target sequence chosen from the HBsAg region of HBV as represented in Figure 1.

5. Method according to claim 2 for detecting the presence or absence of one or more lamivudine resistant HBV strains in said sample, wherein step (iii) consists of hybridizing with at least two nucleotide probes hybridizing specifically to a target sequence chosen from the HBV RT pol gene region as represented in Figure 1.

6. Method according to any of claims 1 to 5, wherein step (iii) consists of hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from Figure 1.

7. Method according to any of claims 1 to 6, wherein step (iii) consists of hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the preCore region as represented in Figure 1.

8. Method according to any of claims 1 to 6, wherein step (iii) consists of hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1 and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence within the HBsAg region as represented in Figure 1.

9. Method according to any of claims 1 to 8, wherein step (iii) consists of hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence chosen from the preCore region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence chosen from the HBsAg region as represented in Figure 1.

10. . Method according to any of claims 1 to 9, wherein step (iii) consists of hybridizing with at least one nucleotide probe hybridizing specifically to a genotype specific target sequence chosen from Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV mutant target sequence within the preCore region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a HBV vaccine escape mutant target sequence chosen from the HBsAg region as represented in Figure 1, and at least one nucleotide probe hybridizing specifically to a lamivudine resistant HBV mutant target sequence chosen from the RT pol gene region of HBV, as represented in Figure 1.

11. Method according to any of the preceding claims wherein the genotype

specific target codons are within the preS1 region of Figure 1.

12. Probe suitable for hybridizing in a method as defined in any of claims 1 to 11.

13. Combination of at least two probes according to claim 12.

14. Use of a probe according to claim 12 for diagnosing or monitoring HBV genotypes and/or HBV mutants present in a biological sample.

15. Use of a combination of probes according to claim 13 for diagnosing or monitoring HBV genotypes and/or HBV mutants present in a biological sample.

16. Assay kit for diagnosing or monitoring HBV genotypes and/or HBV mutants present in a biological sample comprising the following components :

(i) when appropiate, a means for releasing, isolating or concentrating the polynucleic acids present in said sample;

(ii) when appropriate, at least one suitable primer pair;

(iii) at least two of the probes according to claim 13, possibly fixed to a solid support;

(iv) . a hybridization buffer, or components necessary for producing said buffer;

(v) a wash solution, or components necessary for producing said solution;

(vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.

(vii) when appropriate, a means for attaching said probe to a known location on solid support.

5

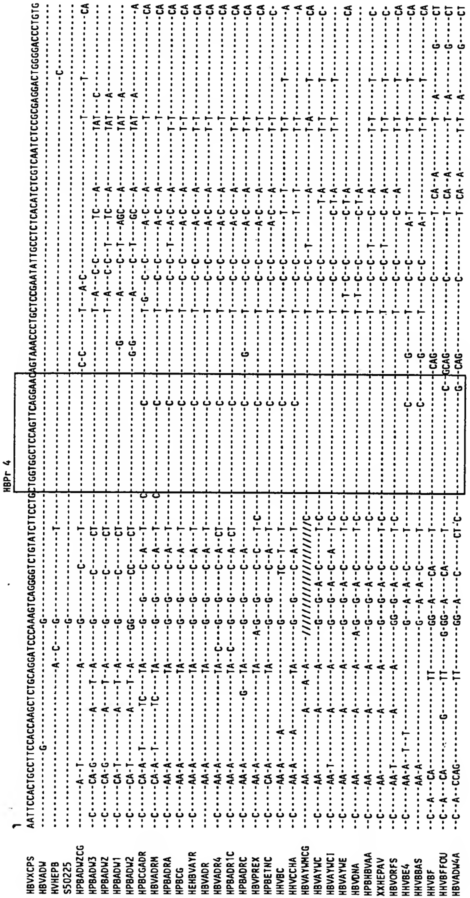

Figure 1

```
                   Start HBsAg
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HBVADW     ----T-  -----------------------------------------------------------------------------------------------------------------------------------A------------
HVHEPB     ------  -------------------------------------C---------------------------------------------------------------------------------------------A------------
S50225     ------  ----------------------------------------------------------C-----------------------------------------------------------------------------------C
HPBADWZCG  C-----  -----------------------------------------------------------------------------------------------------------------------------------AG-----------
HPBADW3    C-----  ------------G--------C---------------------------------------C--------A---------------T-T-----------------------------------------AAA-----------
HPBADWZ    C-----  ------------G--------C------------------------------------------------A---------------A--------------------------------------------AA-----------
HPBADW1    C-----  ------------G--------C------------------------------------------------A---------------A--------------------------------------------AA-----------
HPBADW2    C-----  ------------G--------C------------------------------------------------A---------------A--------------------------------------------AA-----------
HPBCGADR   C-----  -------G--CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HBVADRM    C-----  -------G--CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HPBADRA    C-----  -------G--CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HPBCG      C-----  -------G--CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HEHBVAYR   C-----  -------G--CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HBVADR     C-----  ----------CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HBVADR4    C-----  ----------CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HPBADR1C   C-----  ----------CA--------------------------------------------------------------------------A---------C---------------------------------CAG-----AC----
HPBADRC    C-----  ----------CA--------------------------------------------------------------------------A--------------------------------------------AG-----AC----
HBVPREX    C-----  -------G--CA--------------------------------------------------------------------------A-------------------T------------------------AA-----AA----
HPBETNC    C-----  ----------CA--------------------------------------------------------------------------A----------------///-------------------------AG-----AC----
HHVBC      ------  ----------CA----------------------------------------------------------A---------------A--------------------------------------------AG-----------
HHVCCHA    ------  ----------CA--------------------------------------------------------------------------A---------------G---------------------------AAG----AAG----
HBVAYWMCG  CT----  -----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVAYWC    CT----  -----------------------------------T-----------------------------------------------------------------------------------------------AA-TA--------
HBVAYWCI   -----T  ----------------------------------------------------------------------------------------------------------------------------G------AA-TA--------
HBVAYWE    CT----  -----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVDNA     ----T-  ---------------------------------------------G-------------------------------------------------------------------------------------AA-TA--------
HPBHBVAA   CT----  ----------------------------------------------------------------------A----------------A-------------------------------------------AA-CA--------
XXHEPAV    CT----  -----------------------------------------------------------------------------------------------------------------------------------AA-TA--------
HBVORFS    CT----  -----------------------------------------------------------------------------------------------------------------------------------AA-CA--------
HHVBE4     C-----  -----A-G--------------------------------------------------------------A--------------------------------------------------------------AG-T---------
HHVBBAS    C-----  -----A-G--------------------------------------------------------------A--------------------------------------------------------------AG-T---------
HHVBF      -T----  ---------------------C---------------G--------------T--G--------------A---------------A--------------------------------------------ACT----AG----
HHVBFFOU   -T----  -----C---------------C------------------------------T--G--------------A---------------A--------------------------------------------ACT-----G----
HBVADW4A   -T----  -----C-----T---------C------------------------------T--G--------------A---------------A--------------------------------------------ACT-----G----
```

```
           301
HBVXCPS    -CTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTG
HBVADW     ------------------------------------------------------------------------------------------------------------------------------------------------------
HVHEPB     ----------------GA-----------------------------------------------------------------------------------------------------------------------A------------
S50225     -----------------------------------------------------------------------------------------------------------------------------------------A------------
HPBADWZCG  -----------------------------------------------------------------------------------------------------------------------------------------A------------
HPBADW3    ----------------------A--T-----G------------T-GT--------G--------------------------------------------C-------G----------------------------------------
HPBADWZ    ----------------------A--T-----G------------T-GT--------G--------------------------------------------C-------G----------------------------------------
HPBADW1    ----------------------A--T-----G--------------GT-----------------------------------------------------C-------G----------------------------------------
HPBADW2    ----------------------A--T-----G--------------GT-----------------------------------------------------C-------G----------------------------------------
HPBCGADR   -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVADRM    -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HPBADRA    -C---------------------------------------------T------------------------------------------------------------------------------------------------------
HPBCG      -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HEHBVAYR   -C---------------------------------------------T----------C---------C---------------------------------------------------------------------------------
HBVADR     -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVADR4    -C---------------------------------------------T--------------------C---------------------------------------------------------------------------------
HPBADR1C   -----------------------------------------------T--------------------------T---------------------------------------------------------------------------
HPBADRC    -C-----C---------------------------------------T--------------------C---------------------------------------------------------------------------------
HBVPREX    -C---------------------------------------------T----------C---------C---------------------------------------------------------------------------------
HPBETNC    -C---------------------------------------------T--------------------C-------------------------------------------------------------------------C-------
HHVBC      -C---------------------------------------------T--------------------C--------------------------------C------------------------------------------------
HHVCCHA    -C---------------------------------------------T--------------------C--------------------------------C------------------------------------------------
HBVAYWMCG  ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVAYWC    ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVAYWCI   ----------------------------------------------------------C-------------------------------G----------C------------------------------------------------
HBVAYWE    -----------------------------------------------T----------C------------------------------------------C------------------------------------------------
HBVDNA     ------------------------------C-----------------------------------------------------------------------------------------------------------------------
HPBHBVAA   ----------------------------------------------------------C------------------------------------------C------------------------------------------------
XXHEPAV    ----------------------------------------------------------C------------------------------------------C------------------------------------------------
HBVORFS    ----------------------------------------------------------C------------------------------------------C-----------------T--------------------------A---
HHVBE4     -----------------------------------------------T--------------------C--------------------------------C------------------------------------------------
HHVBBAS    -------------------------------G---------------T--------------------C--------------------------------C------------------------------------------------
HHVBF      -C-----------------------------------T--------------------C---------C-----T--------------------------C------------------------------------------------
HHVBFFOU   -C-----------------------------------T--------------------C---------C-----T--------------------------C------------------------------------------------
HBVADW4A   -C-----------------------------------T--------------------C---------C-----T--------------------------C------------------------------------------------
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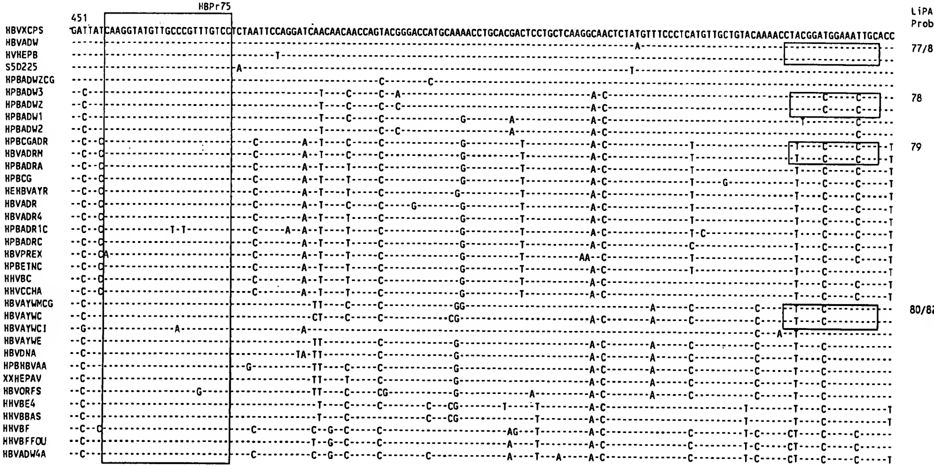
HBPr75
451
LiPA Probe
77/81
78
79
80/82
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HBVXCPS
HBVADW
HVHEPB
S5D225
HPBADWZCG
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HPBADWZ
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWCI
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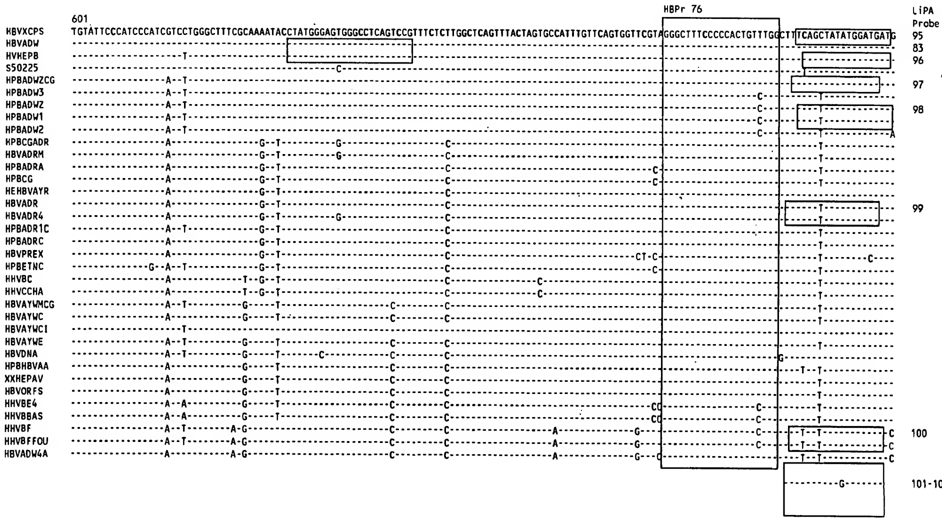
HBPr 76
LiPA
Probe
601
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HBVXCPS
HBVADW
HVHEPB
S50225
HPBADWZCG
HPBADW3
HPBADWZ
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWCI
HBVAYWE
HBVDNA
HPBHBVAA
XXHEPAV
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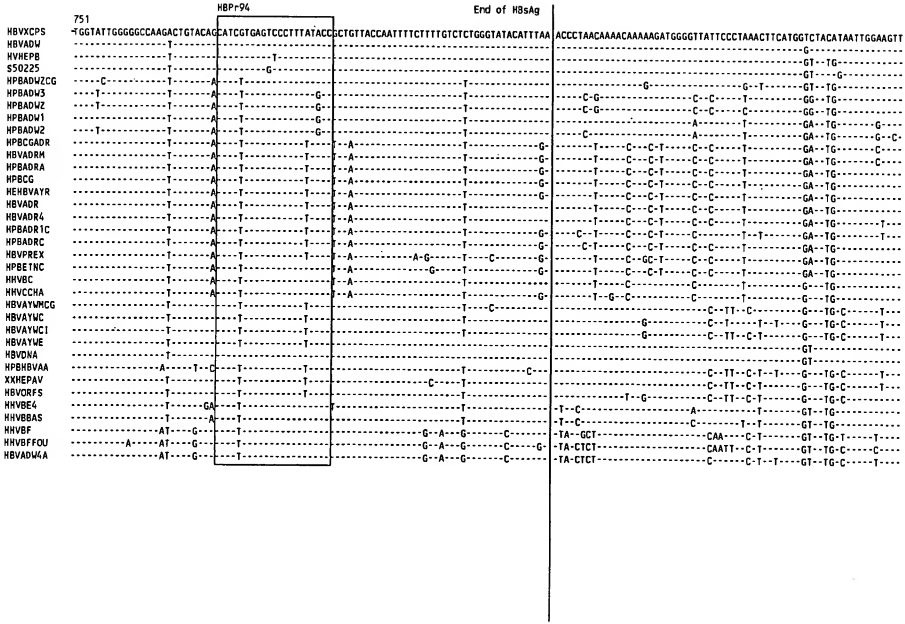
97
98
99
100
101-103



HBPr94
End of HBsAg
751
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HBVXCPS
HBVADW
HVHEPB
S50225
HPBADWZCG
HPBADW3
HPBADWZ
HPBADW1
HPBADW2
HPBCGADR
HBVADRM
HPBADRA
HPBCG
HEHBVAYR
HBVADR
HBVADR4
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWCI
HBVAYWE
HBVDNA
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XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A

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HBVXCPS    GGGGAACATTGCCACAGGATCATATTGTACAAAAGATCAAACACTGTTTTAGAAAACTTCCTGTTAACAGGCCTATTGATTGGAAAGTATGTCAAAGAATTGTGGGTCTTTTGGGCTTTGCTGCTCCATTTACTCAATGTGGATATCCTG
HBVADW     -------T-----------------------------------------------------------------------------------------------------------------------------A----------------
HVHEPB     ----------------------------------A--------------------------------------------------------------------------------------------T-----A----------------
S50225     ----------------------C--------------------------------------------------------------G-----------------------------------------T-----A----------------
HPBADWZCG  ----T-----------------------------A--------------------------------TC-A-----------------------G---C----A--------A--------C-----------A--------T--C----
HPBADW3    ----G--C--A--C--A--A--------GTTG--A--------A--------G-----------A------------------------------C-------------------A-----------T--C--A----------------
HPBADWZ    ----C--C--A--C--A--A--------GTTG--AC-------A--C-----------------A-----A--------------G--G------C-------------------A-----------T--C--A--------T-------
HPBADW1    ----C----G------------------------CT------TA--------------------A-----------------------T------C-------------------G--------C--T-----G----------------
HPBADW2    ----C--------------A--------------A------ATG--G-----G-----------A------------------------------C-------------------G-----C--C--T--C--G----------------
HPBCGADR   ----T--T--A--G--A--A----------T---A-----G--A------C-G-----G-----A--T--A--------C--------------------------------------------C--T-----A--------C-------
HBVADRM    ----T--T--A--G--A--A----------T---A-----G--A------C-G-----G-----A--T--A--------C--------------------------------------------C--T-----A--------C-------
HPBADRA    ----T--T--A--------A---------TT---AC----G--A------C-------G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HPBCG      ----T--T--A--------A---------TT---AC----G--A------C-G-----G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HEHBVAYR   ----T--T--A--G-----A--------------AC----G--A------C-----T-G-----A--T--A-----------------------------------------------------C--T-----A--------C-------
HBVADR     ----T--T--A--G-----A----------T---AC----G--A------C-----T-G-----A--T--C-----------------------------------------------------C--T-----A-----C--C-------
HBVADR4    ----T--T--A--G--A--A----------T---A-----G--A------C-------G-----A--T--A-----------------------G---C-------------------------C--T-----A--------C-------
HPBADR1C   ----T--T--A--G--A--A----------T---A-----G--A------C-------G-----A--T--A-----------------------G-----------------------------C--T-----A--------C-------
HPBADRC    ----T--T--A--------A----------T---A-----G--A------C-G--G--G-----A--T--A--------------------------G--------------------------C--T-----A--------C-------
HBVPREX    ----T--T--A-----A--A--------------AC----G--A------C-------G-----C--T--A-----------------------G-----------------A-----------C--T-----A--------C-------
HPBETNC    ----T--G--A--------A--------------A-----G--A------C-G-----G-----A--T--A-----------------------------------------------------C--T-----A--------T-------
HHVBC      ----T--C--A-----A--A----------T---A----C---A------C----------------T--------------------G-----------------------------------C--T-----A--------G-------
HHVCCHA    ----T--C--A-----A--A----------T---A--------A------C-------------A--T-----------------G--C--C-----------------------A--------C--T-----A----------------
HBVAYWMCG  AT--GT----------A-----C--CAG---G--A------G-A-------------------------------------------GC------C-----------T-A-----T--------C--T-----A--------T-------
HBVAYWC    AT--GT-C--------A--A--C--CA-------A------G-A---------------------------------------------------C-------------------T--------C--T--------------T-------
HBVAYWCI   AT--GT-C--------A--A--C--CA-------A------G-A------------G--------------------------------------C----------------A--T--------C--T--C--A--------T-------
HBVAYWE    -------G--------A------------------------G-A-----------------------------------------------G---C-------------------------------------A----------------
HBVDNA     -------G-----------------------------------------------------------------------------------G---C-------------------T-----------------A--------T-------
HPBHBVAA   AT--GT-T--------A-----C--CA----G--A------G-A--------------------------------------------C------C-T-----------------A-----------T-----A--------T-------
XXHEPAV    AT--GT----------A-----C--CA----G--A------G-A--C-----------------------------------------C------C-T-----------------T--------C--T-----A--------T-------
HBVORFS    AT--GT----------A-----C--CA----G--A------G-A------------------A-------------------------C------C-T-----------------T--------C--T-----A--------T-------
HHVBE4     ----GT----A--------A--C--CA-------A---------------G-------C---------C--------------------------G----------------------------C--T-----A--------G-------
HHVBBAS    ----CT----A--------A--C--CA-------A------G----------------C---------C-------------------------------------------------------C--C-----A----------------
HHVBF      -------G--A--C--A--------------C--A------G-T------C-----G-------A--TC-C--A--------------T--------T-----------------------G--C--T-----C--------T-------
HHVBFFOU   -----G----A--C--A--------------C--A------G-A------C-----G-------A--TC-T--A-----C--------T------C-T--------A--------------------T-----C--------T-------
HBVADW4A   -----T----A--C--A-----C--------C--A-----GG-A--C---C-------G-----A--TC-T--A--------------T------C-C--A-----------------------C--T--C--C-----C--T-------
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HBVXCPS    CCTTAATGCCTTTGTATGCATGTATACAAGCTAAACAGGCTTTCACTTTCTCGCCAACTTACAAGGCCTTTCTAAGTAAACAGTACATGAACCTTTACCCCGTTGCTCGGCAACGGCCTGGTCTGTGCCAAGTGTTTGCTGACGCAACCC
HBVADW     ------------------------------------------------------------------------------------------------------------------------------------------------------
HVHEPB     ----------C-----------------------------------------------------------------------------------------------------------------------A--------T-------
S50225     --------------------------------------------T-----------------------------------------C---------------------------------------------------------------
HPBADWZCG  -A-----------------------------G-----------T-----------------------------------------T--------------------C-------------------------------------------
HPBADW3    -T-----------A-----------------------------T-----T--------G--T-------------AC-----A--TC-------------------------------A-------------------------------
HPBADWZ    -T-----------A-----------------------------T-----T--------G--T-------------CC-----A--TC-------------------------------A-------------------------------
HPBADW1    -T-----------A-----------------A-----------T-----------------------------C-----------T----C-----------------------------------------------------------
HPBADW2    -T-----------A-----------------A-----------T-------------------------------C---------TC-------------------------------A-------------------------------
HPBCGADR   -------------A---------------T----G--------------------------------------GT-------A--TC--C----------------C----G-AC-G--C///-C--------A----------------
HBVADRM    -------------A---------------T----G--------------------------------------GT-------A--TC--C----------------C----G-AC-G--C///-C--------A----------------
HPBADRA    ----G------------------------T----G--------------------------T-----------GT--C----A---C--C----------------C---------T-A-----C-------------------------
HPBCG      ----G--------A---------------T----G--------------------------T-----------GT--C----A---C--C----------------C---------T-A-----C-------------G-----------
HEHBVAYR   ----G--------A---------------T----G--------------------------------------GT-------A--TC-A-----------------C---------T-A-----C-------------------------
HBVADR     ----G--------A---------------T----G--------------------------T-----------GT-------A--TC-------------------C---------T-A-----C-------------------------
HBVADR4    -------------A---------------T----G--------------------------------------GT-------A--TC-------------------C---------T-A-----C-------------------------
HPBADR1C   ----G--------A---------------T----G--A-----------------------------------GT-------A--TC-------------------C---------T-A-----C-------------------------
HPBADRC    ----G--------A---------------T----G--------------------------------------GT-------A--TC--C----------------C---------T-A-----C-------------------------
HBVPREX    ----G--------A-----C---------T----G--------------------------------------GT-------A--TC-------------------C---------T-A--C--C-----------------T-------
HPBETNC    ----G--------A--------------------G--------T---------T-------------------GT-------A--TC--C----------------C---------T-A-----C-------------------------
HHVBC      ----------C--------C-----T--------G--------------------------T-----------GT-------A--TC-------------------C---------T-------T-------------------------
HHVCCHA    -------------------------T--------G--A-----------T---T----------A--------GT-------A--TC-------------------C---------T-------C-------------------------
HBVAYWMCG  -G--G--------------------T---T----G--------------------------------------GT-------A---C-------------------C-----------A-------------------------------
HBVAYWC    -T--------C--------------T---T----G--------------------------------------GT-------A---C-------------------CG----------A-----A-------------------------
HBVAYWCI   -T--------C--------T-----T---TT---G--------------------------------------GT-------A---C-------------------C-----------A-----A-------------------------
HBVAYWE    -------------------C----------------------------------------------------------------------------------------------------------------------------------
HBVDNA     -------------------C----------------------------------------------------------------------------------------------------------------------------------
HPBHBVAA   -T--------C--------------T---T----G--------------------------------------GT-------A---C-------------------C--------C--A-------------------------------
XXHEPAV    -T-----------------------T--GT-G--G--------T-----------------------------GT-------A---C-------------------C-----------A-------------------------------
HBVORFS    -T--------C--------C-----T---T-------------------------------------------GT-------A---C--------------------A----------A-------------------------------
HHVBE4     -T---------C-----A-G-----T---T----G--------------------------------------GT-------A---C-------------------C-----------A-----------------------T-------
HHVBBAS    -T---------C------------CT---T----G--------------------------------------GT-------A---C-------------------C-----------A-----------------------T-------
HHVBF      -TC-C--------------C-----TACT--------------TGTC--------------------------GT-------A-----------------------------------A--C----------------------------
HHVBFFOU   -TC-C------C-----AAC-----CACT--G-----------TGTC--T-----------------------CT-------------------------------------------A--C----------------------------
HBVADW4A   -TC-C------C-------C-----TACT--------------TGTC-----------C--------------GT-------A----------------G------------------A--C----------------------------
```

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           1201
HBVXCPS    CCACTGGCTGGGGCTTGGCCATAGGCCATCAGCGCATGCGTGGAACCTTTGTGGCTCCTCTGCCGATCCATACTGCGGAACTCCTAGCCGCTTGTTTTGCTCGCAGCCGGTCTGGAGCAAAGCTCATCGGAACTGACAATTCTGTCGTCC
HBVADW     ----------------A-------------------------------------------------------------------------------------------------------------------------------------
HVHEPB     ----------------------------------------C-----------------------------------------------------------------------------G--A----------------------------
S50225     ----------------A-------------------------------------------------------------------------------------------------------------------------------------
HPBADWZCG  ----------------------C-------------------A---------------------------------A--------------------------------------------A--------G-------------------
HPBADW3    -------------------------------------------G---------T---------------------T------------A------------------A-------------A--T-----G-------------------
HPBADWZ    -----------------------------------------------------T-----------------------------------------------------A-------------A--T-----G--------------T----
HPBADW1    -------T---------------------------------------------T-----------------------------------------------------A-------------C--------G--C--------------A-
HPBADW2    -------T---------------------------------------------T-------------------------------------------------------------G-----A--------G-----------------G-
HPBCGADR   -------A----------------------G---------------------------------------------------------A-----------------------------G--A--------G--------C--G--T--T-
HBVADRM    -------A----------------------G---------------------------------------------------------A-----------------------------G--A--------G--------C--G--T--T-
HPBADRA    -------A----------------------G-----------------------T---------------------------------A---------------GA---------------A--T-----G--------C--G--T----
HPBCG      -------A----------------------G---------------------------------------------------------A--------------------------------A--T-----G--------C-----T----
HEHBVAYR   ----G--T----------------------G---------------------------------------------------------A-----------------------------G--A--T--------C-----C--A--T----
HBVADR     -------A----------------------G---------------------------------------------------------A-----------------------------G--A--T--------C-----C-----T----
HBVADR4    -------A-----------T--C------AGC--------C---/-------------------------------------------A-----------------G-----------G--A--T-----C--C-----C-----T----
HPBADR1C   -------A-----------T--T-------GC-----------------------------------------------------G--A--C-----------------------------A--T--------C-----C-----T----
HPBADRC    -------A--------------T-----ATC-G-------------------------------------------------------A-----------------------------G--A--T-----G--------C-----T----
HBVPREX    -------A--------------T-----------------------------------------------------------------A-----------------------------G-CA--T--------C-----C-----T----
HPBETNC    -------A----------------------G---------------------------------------------------------A-----------------------------G--A--T-----G--------C-----T----
HHVBC      ----------------------G------------------------------------------------------------------G--------------------------------CA-T--------C-----C----------
HHVCCHA    ----------------------T---A-------------------------------------------------------------A--------------------------------A--T--------------C----------
HBVAYWMCG  ------------------T---G---------------------------CG-------------------------------------------------------A-------------CA-TC----G--G--T--C-T---T----
HBVAYWC    -------------------T--G---------------------------TC-------------------------------------------------------A-------------CA-T-----G-----T--C-----T----
HBVAYWCI   ------------------GT--G---------------------------TC---------------------------------------C---------------A-------------CA-TC----G--G--T--C-----T--T-
HBVAYWE    -------------------A------A--------------------A-------------------------------------------------------------------G-----------------------------T----
HBVDNA     -------------------ATAG----------------------------A-------------------------------------------------------A-------------CA-T-----G-----T--C-----T----
HPBHBVAA   ------------------T---G---------------------------CA---------------------------------------------------------------------CA-TC----G--G--T--C-----T--T-
XXHEPAV    ------------------T---G---------------------------C------G-------------------------------------------------A-------------CA-TC----G--G--T--C-----T--T-
HBVORFS    ------------------T---G---------------------------C--------------------------------------------------------A-------------CA-TC----G--G--T--C-----T--T-
HHVBE4     --------------------------AT------------C------------------------------------------------------------------A-------------A--T-----G--C--T-----------T-
HHVBBAS    --------------------------AT------------C------------------------------------------------------------------A-------------A--T-----G--C--T-----------T-
HHVBF      -------------------------------------------------------------------------------------T--A-----C--C----------------------TC--------C--A----------------
HHVBFFOU   -------T--------------T--------------------------------------------------------------T--A-----C--C----------------------TC--------C--A----------------
HBVADW4A   -------T--------------T-----------------------------------------------------------------A--------C---------A----------G-CT--------C--G-----------T----
```

```
                                                    Start of Hbx protein
           1351
HBVXCPS    TCTCGCGGAAATATACATCGTTTCC ATGGCTGCTAGGCTGTACTGCCAACTGGATCCTTCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCGCTGAATCCCGCGGACGACCCCTCTCGGGGCCGCTTGGGAGTCTCTCGTCCCCTTCTCC
HBVADW     -------------------A----- ----------------------------------------------------------------------------------------------------------C------------------
HVHEPB     ----------------C-------- ------A---------G-----------------------------------------------------------------------------------------C------------------
S50225     ------------------------- ----------------G-----------------------------------------------------------------------------------------C---A--------------
HPBADWZCG  -T--T-A------------C----- --A---------T-------------A---T--------------------C--------------------------------------G--A------------C-G-A--------------
HPBADW3    -T--C--C----------------- ----------------G----------------G-----------------C--------------------------------------C--------------GC---AC--C--G-------
HPBADWZ    -T--C--C----------------- ----------------G----------------G-----------------C-----T--------------------------------C--------------GC---AC--C--G-------
HPBADW1    ----C--C--G-------------- ----------------G----------------G--------------------------------------------------------C--------------GC---AC--C--G-------
HPBADW2    ----C--C--G--------A----- ----------------G----------------G-----------------------------------------------------T--C--------------GC---AC--C--G-------
HPBCGADR   ----T--------C--C--A--C-- ---------C--G---G----------------G----------A------------------------------------------G-----C-----T-----CC--ATC-----------T-
HBVADRM    ----T--------C--C--A--C-- ---------C--G---G----------------G----------A------------------------------------------G-----C-----T-----CC--ATC-----------T-
HPBADRA    ----T--------C--C--C--C-- ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC------T-G--TT
HPBCG      ----T--------C--C--C--C-- ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HEHBVAYR   ----T--------C--C--C----- ----------------G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HBVADR     ----T--------C--C--C----- ------------G---G----------------G-----------------C-----------------------------------G-----------T-----GC---AC-----------T-
HBVADR4    ----T--------C--C--C----- ------------G---G----------------G-----------------C-----------------------------------G-----------T------C---AC-----------T-
HPBADR1C   ----T--------C--C--C----- ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---A------------T-
HPBADRC    ----T--------C--C--C--C-- ---------C--G---G----------------G-----------------C-----------------------------------G-----------T-----CC---AC-----------T-
HBVPREX    ----T--------C--C--C----- ------------G---G----------------G-----------------C-----------------------------------G-----------T-----CC---A------------T-
HPBETNC    ----T--------C--C--C--C-- ---------C--A---G----------------G-----------------C-----------------------------------G--------T--T-----CC---AC-----------T-
HHVBC      ----T--------C-----C----- ---------C--G---G----------------A-----------------------------------------------------G-----C-----T-----GC---AC-----------TT
HHVCCHA    ----T--C-----C-----C----- ---------C------G----------------A--A--------------------------------------------------G-----------T-----GA---AC-----------T-
HBVAYWMCG  -A--C--C----------------- ----------------G----------------GA-------------C--------------------------A-----------T------------------C------------------
HBVAYWC    ----C--C----------------- ----------------G----------------G-----------------------------------------------------G--------T---------C------------------
HBVAYWCI   ----C--C----------------- ----------------G----------------G-----------------------------------------------------T------------------C-----------------T
HBVAYWE    ------------------------- ------------T---------------------------------------------------------------------------T------------------C------------------
HBVDNA     -A----------------------- ------------T---------------------------------------------------------------------------------------------C------------------
HPBHBVAA   ----C--C--------G-------- ----------------G----------------G-----------------------------------------------------T------------------C------------------
XXHEPAV    ----C--C-------------A--- ----------------G----------------G-----------------------------------------------------T--------T---------C------------------
HBVORFS    ----C--C----------------- ----------------G----------------G-----------------------------------------------------T-----------------GA---T--------------
HHVBE4     ----C--------------C----- ----------------G----------------G--A--------------C-----------A-----------T-----------G--------T--------GA---T--------------
HHVBBAS    ----C-----G--------C----- ----------------G----------------G--A--------------C-----------A-----------T-----------G--------T--------GA---A--------------
HHVBF      ----T-----G--------C----- ---------C--T---G----------------G-----------------------------------------A--------A-----C-----T--------GC-G-AC--C--------T-
HHVBFFOU   ----C-----G--------C----- ---------C--A---G----------------G-----------------------------------------A--------A-----C--------------GC---AC--C--T-----G-
HBVADW4A   ----TA----G--C--C--C----- ---------C--A---G----A-----------G--------------------------A-----------------------------C--------------GC-G-AC--C--T-------
```

certified copy of EP priority document obtained from PTO IFW relating to Appln. No. 10/453,792


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                                                                                                              End of HB pol protein
            1501
HBVXCPS     GTCTGCCGTTCCAGCCGACCACGGGGCGCACCTCTCTTTACGCGGTCTCCCCGTCTGTGCCTTCTCATCTGCCGGTCCGTGTGCACTTCGCTTCACCTCTGCACGTTGCATGGAGACCACCGTGA ACGCCCATCAGATCCTGCCCAAGGT
HBVADW      -----------------------------------------------------------------------------------------------------------------C----------- -------------------------
HVHEPB      ----------------------------------------------------------------------------------------------------------------------------- -------------------------
S50225      ----------------------------------------------------------------------------------------------------------------------------- -------------------------
HPBADWZCG   ---------A--GT--------------------------------------------------------------------------------------------------------------- ------------G------------
HPBADW3     -C-------A--GT-------------------------------A-----------------------------A------------------------------C------------------ -----------G-------------
HPBADWZ     ---------A--GA-------------------------------A--------------------G--------A------------------------------C------------------ ---------G--A------------
HPBADW1     ---------A--GT-------------------------------A-----------------------------A------------------------------C------------------ -------C-G--A------------
HPBADW2     -C---TT--A--GA-------------------------------A-----------------------------A------------------------------C------------------ -------CGG--A------------
HPBCGADR    A-----G-----G--------------------------------------------------------------A------------------------------C------------------ -----G-----G--T----------
HBVADRM     A-----G-----G--------------------------------------------------------------A------------------------------C------------------ -----G-----G--T----------
HPBADRA     C--------------------------------------------------------------------------A------------------------------C------------------ ---G---C---G--T--------C-
HPBCG       A---------------------------------------------------------------------------------------------------------C------------------ -------C---G--T----T---C-
HEHBVAYR    A-----------G--------------------------------------------------------------A------------------------------A------------------ -------C---G--T----------
HBVADR      T-----------G--------------------------------------------------------------A------------------------------C------------------ -------C---G--T----------
HBVADR4     A-----------G-----------------------------------TTTT------------------------------------------------------C------------------ -------C---G--T----------
HPBADR1C    A---A-------G--------------------------------------------------------------A-------------C----------------C------------------ -------C---G--T----------
HPBADRC     A-----------G----------------G----------------------------------------------------------------------------C------------------ -------C---G--T----------
HBVPREX     -----T------G--------------------------------------------------------------A------------------------------C------------------ -------C---G--T----------
HPBETNC     A-----------G--------------------------------------------------------------A------------------------------C------------------ -----------G-GT----------
HHVBC       ------G-----G---A----------------------------------------------------------A------------------------------C-------A---------- -------CAT-G--T----------
HHVCCHA     ------G-----G---A----------------------------------------------------------A------------------------------C------------------ -------CAT-G-AT----------
HBVAYWMCG   ----------T-GT-------------------------------A-----------------------------A------------------------------C------------------ -------C--AT--T----------
HBVAYWC     ------------GA-------------------------------A---------------------------T-A--T---------------------------C------------------ -------C--A--AT----------
HBVAYWCI    ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ -------------------------
HBVAYWE     --------------------------------------------------------------------------------------------------------A-------------------- -------------TA----------
HBVDNA      -------------------------------------------------A--------------------------------------------------------C------------------ ----------A-GT-----------
HPBHBVAA    ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ -------C--AT--T----------
XXHEPAV     ----------T-GA-------------------------------A-----------------------------A------------------------------C------------------ -A-----A-CAT--T----------
HBVORFS     ------------GT-------------------------------A------------------------------------------------------------C------------------ -------C--CT--T----------
HHVBE4      ------G-----G--------------------------------------------------------------A------------------------------C------------------ -------C--A---T----------
HHVBBAS     ----------------------------------------------------------T----------------A------------------------------C------------------ -------C--A--AT----------
HHVBF       -------------------A-----T-------------------A------------T----------------A------------------------------C------------------ ------CCTG--GTT----A-CA--
HHVBFFOU    -------------------------T-------------------A------------T-----------------------------------------------C------------------ ------CCTG--GTT----A-CA--
HBVADW4A    -------------------G-----T-------------------A------------T----------------A------------------------------C------------------ ------CCTG--GTT----A-CA--
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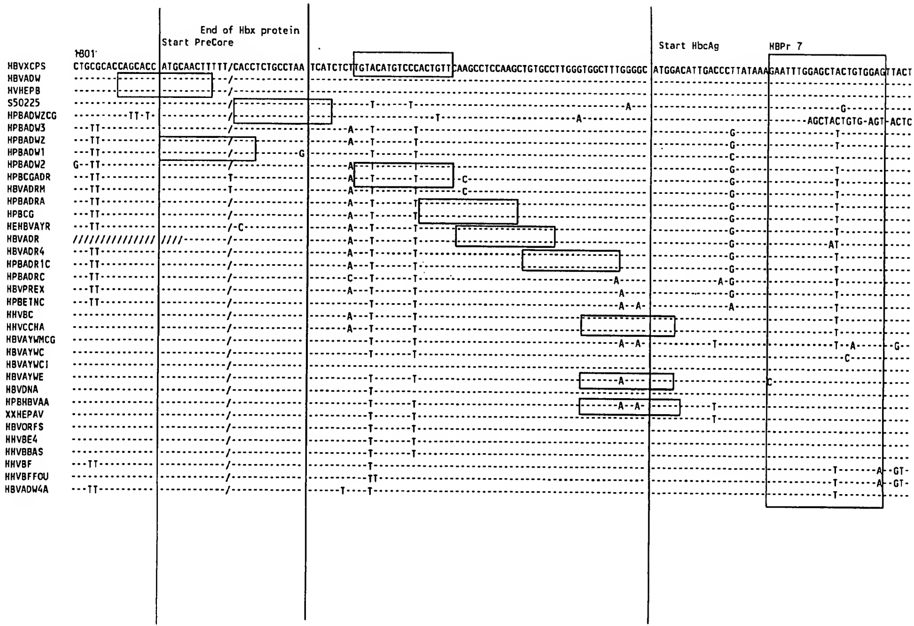
                HBPr 69                HBPr 70                                                                        LiPA
          1651                                                                                                        Probe
HBVXCPS   CTTACATAAGAGGACTCTTGGACTCTCAGCAATGTCAACGACCGACCTTGAGGCCTACTTCAAAGACTGTGTGTTTAAGGACTGGGAGGAGCTGGGGGAGGAGATTAGGTTAAAGGTCTTTGTATTAGGAGGCTGTAGGCATAAATTGGT
HBVADW    -------------------------C-----------------------------------------------------------------T---------------------T-A----------------------------------
HVHEPB    -------------------------C----------------------------------------------------------------------------------------------------------------------------
S50225    ------------A------------C---------------------------------------------------------------------------------------T-A----------------------------------
HPBADWZCG ------------------------------------------------------------------------------A------------T----------------------------------------------------------
HPBADW3   ---G--------------------T-----------------------------AC---------------------CT--G---------T-------------C------------------C-------------------------
HPBADWZ   ---G--------------------T------------C----------------A----------------------CT--G---------T-------------C------------------C-------------------------
HPBADW1   ---G--------------------T-----------------------------A-----------------------T--G------------------------------------------C-C-----------------------
HPBADW2   ---G--------------------T-----------------------------A-----------------------T--G---------T-----------G--------------------C-------------------------
HPBCGADR  ----------------------------------------T-------------A--------------CT-------A-----------CT---------------------T-A--------C------------------------- 89
HBVADRM   ----------------------------------------T-------------A--------------CT-------A-----------CT---------------------T-A--------C-------------------------
HPBADRA   ---------------------------------------A--------------A---------------T-------A------------T--------------------------------C-------------------------
HPBCG     ------------------------------------------------------A---------------T-------A------------T--------------------------------C------------------------- 88
HEHBVAYR  ------C-----------------------------------------------A---------------T-------A------------T--------------------------------C-------------------------
HBVADR    ------------------------------C-----------------------A-----------------------A------------T--------------------------------C-----------------////////
HBVADR4   ------------------------------G-----------------------A---------------T--------------------T--------------------------------C-------------------------
HPBADR1C  ------------C-----------------------------------------A---------------T--------------------T--------------------------------C-G-----------------------
HPBADRC   ------------------------------G-----------------------A--T------------T-------A------------T---------------------T----------C-------------------------
HBVPREX   ----T-------------------------------------------------A---------------T-------A------------T---------------------T-A--------C-------------------------
HPBETNC   ----T-------------------T-----------------------------A---------------T--------------------T----------AC---------T-A--------C-------------------------
HHVBC     ---G--------A-----------------------------------------A--T-----------------C--A------------T-----------G----A---------------C-------------------------
HHVCCHA   ---G--------------------------G-----------------------A-----------------A-----A------------T----------------A---------------C-------------------------
HBVAYWMCG ------------------------------------------------------A---------------T-------A--G---------T----------------A------T-G--------------------------------
HBVAYWC   ---------------------------T--------------------------A---------------T-------A------------T----------------A---------------C-------------------------
HBVAYWCI  -------------------------C----------------------------------------------------------------------------------------------------------------------------
HBVAYWE   T------------------------C----------------------------------------------------------------------------------------------------------------------------
HBVDNA    -------------------------C----------------------------------------------------------------------------------------------------------------------------
HPBHBVAA  ---------------------------T-T------------------------A---------------T--------------------TC---------------A----T-A--------C-------------------------
XXHEPAV   ---------------------------T-T------------------------A---------------T-------A------------T----------------A-----------------------------------------
HBVORFS   ------------------------------------------------------A---------------T--------------------T----------------A---------------C-------------------------
HHVBE4    ---------------------------T--------------------------A---------------T-------A------------T----------------A---------------C-------------------------
HHVBBAS   ---------------------------T--------------------------A---------------T-------A------------TC---------------A---------------C-------------------------
HHVBF     ------------------------T----G-CG-----T----TGGA-C--A-A----A-------------A--------------------------------C------------------C-------------------------
HHVBFFOU  ----------------A-------T----G-CG-----T----TGGA-C--A-AA---A-------------A-----A--------------------------C------------------C-------------------------
HBVADW4A  ----------C-------------T----G-TG-----T----TGGA-C--A-AA---A-------------A------------------T----------------------------------------------------------
```



End of Hbx protein
Start PreCore
Start HbcAg
HBPr 7
LiPA Probe
1801
CTGCGCACCAGCACC ATGCAACTTTTT/CACCTCTGCCTAA TCATCTCTTGTACATGTCCCACTGTTCAAGCCTCCAAGCTGTGCCTTGGGTGGCTTTGGGGC ATGGACATTGACCCTTATAAAGAATTTGGAGCTACTGTGGAGTTACT
HBVXCPS 45
HBVADW 12
HVHEPB
S50225 13
HPBADWZCG
HPBADW3
HPBADWZ 34
HPBADW1
HPBADW2 46
HPBCGADR
HBVADRM
HPBADRA 47
HPBCG
HEHBVAYR 48
HBVADR
HBVADR4 49
HPBADR1C
HPBADRC
HBVPREX
HPBETNC
HHVBC 9
HHVCCHA
HBVAYWMCG
HBVAYWC
HBVAYWC1
HBVAYWE 10
HBVDNA
HPBHBVAA 42
XXHEPAV
HBVORFS
HHVBE4
HHVBBAS
HHVBF
HHVBFFOU
HBVADW4A
AGCTACTGTG-AGT-ACTC

HBPr 8

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BVXCPS      CTCGTTTTTGCCTTCTGACTTCTTTCCTTCCGTCAGAGATCTCCTAGACACCGCCTCAGCTCTGTATCGAGAAGCCTTAGAGTCTCCTGAGCATTGCTCACCTCACCATACTGCACTCAGGCAAGCCATTCTCTGCTGGGTGGAATTGAT
HBVADW      ---------------------------------AC--------------------------------------------------------------------------------------------------------G---------
HVHEPB      ---------------------------------------------------G-----------G-------------------------------------------C----------------------------------G---------
S50225      -----------------CA--------------------------------------------------------------------------------------------------------G---------------G------A--
HPBADWZCG   TCAT----GC-T-CTGACT-CT--C-T-C-GTC-G-GATCTA-TAGA-T--A----------A-----G----------------------------------------A-------------------------------G-A----A--
HPBADW3     ---T-------------------------TA-TC-------T--C----------T----------G--G-------------G--A-----T----------------G--------------T-----G--T----GT--G-----
HPBADWZ     ---T-------------------------TA-TC-------T--C----------T----------G--G-------------G--A-----T----------------G--------------T---T-G--T----GT--G-----
HPBADW1     ---T---------------------G--G--GC-G--C---------T----T---T---------G----------A-A--------------------C--A--------------T-----G------------G------A--
HPBADW2     ---T---------------------G--G--GC-----------C----------T---T------G--G-------------G--A-----T----------------G--------------T-----G--T----GT--G--A--
HPBCGADR    ---T-----------T--------------A-TC----------C----------T--------A-G--G-------------G--A-----T--------T-----A--------------T-----G--T----GT--G-----
HBVADRM     ---T-----------T--------------A-TC----------C----------T--------A-G--G-------------G--A-----T--------T-----A--------------T-----G--T----GT--G-----
HPBADRA     ---T-------------------------TA-TC----------C----------T----------G--G-------------G--A-----T----------------A--------------T-----G--T----GT--G-----
HPBCG       ---T-------------------------TA-TC----------C----------T----------G--G-------------G--A-----T----------------A--------------T--C--G--T----GT--G-----
HEHBVAYR    ---T-------------------------TA-TC----------C----------T----------G--G-------------G--A-----T----------------A--------------T-----G--T----GT--G-----
HBVADR      ---T---------------------G--TA-TC----------T----------T----------G--G-------------G--A-----T----------------A--------------T-----G--T----GT--G--A--
HBVADR4     ---T-------------------------TA-TC----------C-------------A------G--G-------------G--A-----T--T-----T-----A--------------T-----G--T----GT--G-----
HPBADR1C    ---T-------------------------TA-TC----------C---------------------G--G-------------G--A----------------------C--------------T-----G--T---CGT--G-----
HPBADRC     ---T-------------------------TA-TC----------C---------TT------C---G--G-------------G--A-----T----------------A--------------T---G-G--T----GT--G-----
HBVPREX     ---T---------------------G--T--TC----------C----------T-----C----G--G-------------G--A-----T----------------A--------------T-----G--T-----T--G-----
HPBETNC     ---T-------------------------TA-TC----------C----------T----------G--G---C----------G-----------TA---------------A--------------T-----G--T----GT--G-----
HHVBC       ---T-----------T--------A--TA-TC----C-----C----------------------G--G-------------G-----------T----------------A--------------TG----G--T----GT--G--A--
HHVCCHA     ---T-----------T--------A--TA-TC----C-----C----------------------G--G-------------G--A-----T----------------A--------------TG----G--T----GT--G--A--
HBVAYWMCG   ----------------------A------A--AC-------T-----T------------A-----G------------------------TA-------T--------------------A-----T-------G------A--
HBVAYWC     -----------------------------A--AC-------T-----T------------------G--T-------------------------T-----------------------------A-----T-------G----C-A--
HBVAYWCI    -------------------------------AC-------------------------------G-----------------------------T-----------------------------A-----T-------G----C-A--
HBVAYWE     -C---A--------------------TC-A--AC-------------T------------------G-----------------------------T-----------------------------A-----T-------G----C-A--
HBVDNA      ---------------------------------A------------------------------------------------------------------------------------------------------G----C----
HPBHBVAA    -------C----------------------A--AC-------T-----T------------A-----G-----------A--------------T-----------------------------A---------------G---TC-A--
XXHEPAV     ------------------------------A--AC-------T-----T-A----------------G-----------------------------T-----------------------------A--A--G-------G----C-A--
HBVORFS     ---A-----------T---------------G--AC-------T-----T-----------------G--T-------------------------T-----------------------------A-----T-------G----C-A--
HHVBE4      ------------------------------A--A--------T-----T-----------------G--T----------A--------A-----T-----G-----C------------------T-------G----C-A--
HHVBBAS     ------------------------------A--A--------T-----T-----------------G--T-----------------------------T-------------C------------------T-------GA---C-A--
HHVBF       ---T-----G----------------G--T--TC-G--C-----C-------------C-----C--G--T----------A--G--A------A-C--CA-T-----C--T-----------T---T-G-------GT--G--A--
HHVBFFOU    ---T----------------------G--AA--C----C--T--C------------------G--T--G---------A--G--A------A-C--CA-T-----C--T-----------T---T-G--T----GT--G--A--
HBVADW4A    ----------------T-----C--A--G--TC-G--C--A--C---------T--------T--C--G--T--T---------A-----A-------A-T--CA----------T------------T---T-G--T----GT--G--A--
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	2101
HBVXCPS	GACTCTAGCTACCTGGGTGGGTAATAATTTGGAAGATCCAGCATCCAGGGATCTAGTAGTCAATTATGTTAATACTAACATGGGTTTAAAGATCAGGCAACTATTGTGGTTTCATATATCTTGCCTTACTTTTGGAAGAGAGACTGTGCT
HBVADW	-------------------------------C----------------A--A--
HVHEPB	--A-----------------C----------G---C----A------------------A-----A--
S50225	-------------------------------C---------------------A---T----TG-----------C-----A--
HPBADWZCG	--CC--------------T----------------T-----------------------A-----C--
HPBADW3	--A------C-----------A-G------------C-----C---C----AT---------G------C---GT---T-----CC----A-----A-----------------C--T--C--T-----G-----------A-----T--
HPBADWZ	--A------C-----------A-G------------C---------C----AT---------G------C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HPBADW1	--C-------------------------C-----------CC------------------G---------C--T--C--T-----------------A-----T--
HPBADW2	--A------C-----------A-G---------------G-----------AT---------GC-----C--CGT---T-----CC----A-----A-----------------C--T--C--T-----------G-----A-----T--
HPBCGADR	--A---G--C-----------A-G------------C--------------A----------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HBVADRM	--A---G--C-----------A-G------------C--------------A----------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HPBADRA	--A-T-G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A--------------------T--C--T-----------------A-----T--
HPBCG	--A-T-G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HEHBVAYR	--A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A--T--A-----------------C--T--C--------------------A-----C--
HBVADR	--A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--------------------A-----TT-
HBVADR4	--A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A------C----------C--T--C--T-----------------A-----T--
HPBADR1C	--A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T--C--CC----A-----A------C----------C--T--C--T-----------------A-----T--
HPBADRC	--A---G--C-----------A-G------------C--------------AT-G-------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A--G--T--
HBVPREX	--A---G--C-----------A-G------------C--------------AT---------GC-----C---GT---T-----CC----A-----A-----------------C--T--C--T-----------------A-----TT-
HPBETNC	--AC--G--C-----------A-G----------------A----------AGC--------GC-----C---GT---T-----CC----AC----A-----------------C--T--C--T-----------------T-----T--
HHVBC	--A---G--------------A-G------------C--------A--A--AT-G-------G------C---GT---T-----CC----A-----------G--------------T--C--T-----------------A-----T--
HHVCCHA	--A---G--------------A-G------------C--------------AT----G----G------C--C-T---T-----CC----A-----A-----------------C--T--C--T-----------------A-----T--
HBVAYWMCG	---------C------------GG-------C--------A----------C----------G---------C-----T-----CC-----T----------------------CG-T-----T--C--------------A--A--CG-
HBVAYWC	----------------------GT---------------------T-----C----------G------C--C-----T-----CC-----T----A-----C-----------C--T-----T--C-T------------A--A--TA-
HBVAYWCI	----------------------GT---------------------T--A--C----------G------C--C-----T-----C------T----------C-----------C--T-----T--C--------------A--A--TA-
HBVAYWE	----------------------GT---------------------T--A--CT---------G------C--C-----T-----CC-----T----------C-----------C--T-----T--C--------------A--A--TA-
HBVDNA	---------AT-----------G-------------------G--T-----C----------G---------C-----T-----CC---A--
HPBHBVAA	A------T-C------------GG----------------A----T-----C----------G---------C-----T-----CC-----T----------------------C--T-----T--C--------------A--G--CA-
XXHEPAV	----------------------GG----------------AT---------C----------G------C--C-----T-----CC----AT----------------------C--T-----T--C--------------A--A--TA-
HBVORFS	----------------------GT---------------------T-----C----------G------C--C-----T-----CC-----T----------------------C--T-----T--C--------------A--A--CA-
HHVBE4	----------------------GTA--------------------------C----------G------C--------T-----CC-----T--------T-------------C--T-----T--C--------------A--C--CA-
HHVBBAS	----------------------GTA--------------------------C----------G------C--------T-----CC-----T--------T-------------C--T-----T--C--------------A--C--CA-
HHVBF	----T-G---T-------------------------C--T---G-T------T-------T--------C--C-----T-----C--------T--A-----------------C--C--C--T-----------------A--A--T--
HHVBFFOU	----T-G---T----------C-----------G--C--T---G--------T-------T--C--------C-----T-----C--------T--A-----------------C--T--C--------------------A--A--T--
HBVADW4A	----T-G---T----------C-----------G--C--T---G-T------T-------T--C--------C-----------CC----A--T--A-----G-----------C--T--C--------------------A--A--T--

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                                                                      Start HB pol protein
           2251
HBVXCPS    TGAATATTTGGTCTCTTTCGGAGTGTGGATTCGCACTCCTCCAGCCTATAGACCACCAA|ATGCCCCTATCTTATCAACACTTCCGGAAACTACTGTTGTTAGACGACGGGACCGAGGCAGGTCCCCTAGAAGAAGAACTCCCTCGCCTCG
HBVADW     -----------------------------------------------------------|-------------------------------------------------------------------------------------------
HVHEPB     -----------------------------------------------------------|-------------------------------------------------------------------------------------------
S50225     -----------------------------------------------------------|-------------------------------------------T-T---------C-----------------------------------
HPBADWZCG  ---G-------------------------------------------------------|------------------------------------------------A------------------------------------------
HPBADW3    ------------G-----T-----------------A--------A-------------|------------------------------------------------//////-------------------------------------
HPBADWZ    ------------G-----T-----------------A-----T--A-------------|------------------------------------------------//////-------------------------------A-----
HPBADW1    G-----------A-----T-----------------------T-----C----------|------------------------------------------------//////-------------------------------------
HPBADW2    ------------G-----T-----------------------T--A-------------|----------------------------------------------A-//////-------------------------------------
HPBCGADR   G--G--------A-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HBVADRM    G--G--------A-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HPBADRA    ---G--------G-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HPBCG      ---G--------G-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HEHBVAYR   ---G--------G-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HBVADR     G--G--------A-----T-----------------------C--T--C----------|------------------------------------------------//////-------------------------------------
HBVADR4    ---G--------G-----T-----------------------T--T--C----------|------------------------------------------------//////-------------------------------------
HPBADR1C   ---G--------G-----T-----------------------T--T--C----------|----------------------------------------------//////---------------------------------------
HPBADRC    ---G--------A---G-T----------------------A------C----------|------------------------------------------------//////-------------------------------------
HBVPREX    A--G--------G-----T----------------------TC--T--C----------|--------------------------------------------A---//////-------------------------------------
HPBETNC    ---G--------G-----T---------------------A-C--T--C----------|------------------------------------------------//////-------------------------------------
HHVBC      ---G--C-----G--C--T-----------------------C--T--C----------|----------------------------------------------A-//////-------------------------------------
HHVCCHA    ---G--------G-----T-----------------------C--T--C----------|----------------------------------------------T-//////-------------------------------------
HBVAYWMCG  A--G--------G-----T----------------------A---T-------------|---------------------------G---TG---------------//////-------------------------------------
HBVAYWC    A--G--------G--------------------------------T-------------|----------C----------------G--------------------//////-------------------------------------
HBVAYWCI   A--G--------GG-------------------------------T-------------|----------C----------------G--------------------//////-------------------------------------
HBVAYWE    A--G--------G--------------------------------T-------------|----------C----------------G-------------C------//////-----------------------------------GC
HBVDNA     -----------------------------------------------------------|---------------------------------------------------//////----------------------------------
HPBHBVAA   A--G--------G-----T--------------------------T-------------|---------------------------G--------------------//////-------------------------------------
XXHEPAV    A--G--------G-----T--------------------------T-------------|---------------------------G--------------------//////-------------------------------------
HBVORFS    A--G--------G--------------------------------T-------------|------------------------------------------------//////-------------------------------------
HHVBE4     A--G--------G-----T--------------------------T-------------|---------------------------G-A----------------A-//////-------------------------------------
HHVBBAS    A--G--------G-----T--------------------------T-------------|---------------------------G-A----------------A-//////-------------------------------------
HHVBF      ---G--------G--C--T--------------------A--T--T-------------|----------C----C--------------------------------//////-------------------------------------
HHVBFFOU   ---G--------G--C--T--------------------------T-------------|----------C----C--------------------------------//////-------------------------------------
HBVADW4A   A--G--------G--C--T-----------------------T--T--C----------|----------C----C--------------------------------//////-------------------------------------
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                                                       End of HBcAg
          2401
HBVXCPS   CAGACGCAGATCTCAATCGCCGCGTCGCAGAAGATCTCAATCTCGGGAATCTCAATGTTAG|TATTCCTTGGACTCATAAGGTGGGAAACTTTACTGGGCTTTATTCCTCTACAGTACCTATCTTTAATCCTGAATGGCAAACTCCTTCCT
HBVADW    -------------------------------------------------------------|---------------------C-----------G-------------------------------------------------------
HVHEPB    -------------------------------------------------------------|------------------------------C----------------------C-----------------------------------
S50225    -------------------------------------------------------------|-----------------------------------------------------------------------------------------
HPBADWZCG -------------------------------------------------------------|---------------------------T-----------------T-----T--C--------------------------A-----T-
HPBADW3   ------A--G---------------------------------------------------|---------------------------------G-----------T-----G-------G----------C----------------A-
HPBADWZ   ------A--G-----------CG--------------------------------------|---C-----------------------------G-----------T-----G-------G----------A------------------
HPBADW1   ------A--G--------A--------------------------------C---------|---C-----------------------------G-----------T------------G----------------------------T-
HPBADW2   ------A--G---------------------------------------------------|------------A--------------------G-----------T-----G------TG----------A----------------T-
HPBCGADR  ------A------G-----------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HBVADRM   ------A------G-----------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HPBADRA   ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G--C----------G-CC--------C----
HPBCG     ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------G--C--------C----
HEHBVAYR  ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------T---A-------C----
HBVADR    ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G----------C--G-----------C----
HBVADR4   ------A--G---------------------------------------------------|---C-----------------------------------------T-----T------G-------------G-----------C----
HPBADR1C  ------A--G---------------------------------------------------|---C-----------------------------------------T-----T--------------------G-----------C----
HPBADRC   ------A--G---A-----------------------------------------------|---C--------------------------------T--C-----T-----T------G-------------G-----------C----
HBVPREX   ------A------------------------------A-----------------------|---C--------------------------------A--------T-----T------G-------------G---A-------C--T-
HPBETNC   ------A--G---------------------------------------C----------A|-G-C-----------------------------A--A-----C--T-----T------G-------------G-----------C----
HHVBC     ------A--G-----------------------------------------C---------|---C-----------------------------------------T-----T------G-------------------------C--T-
HHVCCHA   ------A--G--------A--------------------------------C---------|---C-----------------------------------------T-----T------G-------------------------C--T-
HBVAYWMCG ------A--G---------------------------------------------------|---------------------------------G-----------T-----T--T---G-------C---C-T---A----A--C--T-
HBVAYWC   ------A--G---------------------------------------------------|------------------------G--T-----------------T-----T------G-----------C-T---A----A--A--T-
HBVAYWCI  ------A--G---------------------------------------------------|---------------------------------G---T-------T-----T--T---G-------C---C-T---G----C--C--T-
HBVAYWE   -----CA--G---------------------------------------------------|------------------------G--------G-----------T-----T--T---G-----------C-T---A----A-----T-
HBVDNA    ------A--G---------------------------------------------------|------------------------G--------------------T-----T------G-----------C-T---A----A--A--T-
HPBHBVAA  ------A--G-----------A------------A--------------------------|------C--------------------------G-----------T-----T--T---G-------C---C-T---A----A--C--T-
XXHEPAV   ------A--G---------------------------------------------------|---------------------------------G-----------T-----T------G-------C---C-T---A----A--C--T-
HBVORFS   ------A------------------------------------------------------|------------------A-----T--------G-----------------T------G-------C---C-T---A----A--C--T-
HHVBE4    ------A-------------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----TA-T---G-----------A-C---A-------A--T-
HHVBBAS   ------A-------------------------------------CA-CT--C---------|---------------------------T-----G--------C--T-----TA-----G-----------A-C---A-------A--T-
HHVBF     -C----A--G----------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----T------GCT--C------A-C---TT---------T-
HHVBFFOU  ------A--G----------------------------------CA-CT--C---------|---------------------------T-----G-----C--C--T-----T------GCT--C------C-C---TT---------T-
HBVADW4A  ------A---------------------C-C-------------CA-CT--C---------|---------------------------------G--------C--T-----T--G---GCT---------A-C---TCC--------T-
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          2551
HBVXCPS   TTCCTAAGATTCATTTACAAGAGGACATTATTAATAGGTGTCAACAATTTGTGGGCCCTCTCACTGTAAATGAAAAGAGAAGATTGAAATTAATTATGCCTGCCAGATTCTATCCTACCCACACTAAATATTTGCCCTTAGACAAAGGAA
HBVADW    -------------------------------------------------------------------------------------------------------T----------------------------------------------
HVHEPB    -------A-----------------------------------------------------------------------------------------------T----------------------------------------------
S50225    -------------------------------------------------------------T-----------------------------------------T--------------------A-------------------------
HPBADWZCG -------A-----------T--A------GC------------G--------A-----------------------A------C-------------------T--G--T-------A-AG---A-----------------------G-
HPBADW3   -----G-C--------G--G--------C-------A----A-------------A--C--T--A--G--------A--G---C-A-----G-----------T--G--------C-ATGTT--------------------T-------
HPBADWZ   -----G-C--------G--G-----T--C------------G-------------A--C-----A--G--------C--G---C-A-----G-----------T--G--------C-ATGTT--------------------T-------
HPBADW1   ----AG-C-----------G---------G--G----A---A-G-----------A--C--T--A-----------C--G---C-A--------A--------T-----T-----C-ATGTT--C-----------------T-----T-
HPBADW2   -----G-C--------G--G---------G--G----A---A-G-----------G--C--T--A-----------C--G---C-A--------------C--T--G--T-----C-ATGTT--------------------T-----G-
HPBCGADR  -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G--T-------AA-TT--C--------------G--T-----C-
HBVADRM   -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G--T-------AA-TT--C--------------G--T-----C-
HPBADRA   -------C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----C-
HPBCG     -------C-----------------T-----------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----C-
HEHBVAYR  -----C-C-----------G-----------------A----------A------------G--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--------C-
HBVADR    -----C-C-----------G-----------------A----------A------------T--G--T--------A--G-----A-----------------T--G----------A--TT--------------------------C-
HBVADR4   -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A-----------------T--G----------A--TT--C-----------A--G--------C-
HPBADR1C  -----C-C-----------G-----------------A----------A------------T--A--T--------A--G-----A--G--------------T--G----------A--TT--C------C-------G--------C-
HPBADRC   -------T-----------G-----T-----------A----------A---A--------T--A--T--------A--G-----A-----------------T--G----------A--TT--C--------------G--T-----T-
HBVPREX   -------C-----------G------------G----A----------A------------T--A--G--------A--G-----A-----------------T--G----------A--TT--C--G-----A-----G--T-----C-
HPBETNC   -------C-----------G------------G----A----------A------------T--A--T--------A--G-----A-----------------T--G--T-----A-A--TT--C--------------G--T-----C-
HHVBC     -----G-C--------G--G-----------------A----------A------------T--A--T--------A--------A-----------------T--G--T-------A--TT--------------------------C-
HHVCCHA   -----G-C--------G--G-----------------A----------A------------T--A--T--------A--------A-----------------T--G--T-------A--TT--C-----------------T-----C-
HBVAYWMCG -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G----------T---G--T-----A-TGGTT--C-----------A--G--T--G--T-
HBVAYWC   -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G--C--------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HBVAYWCI  -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G-----------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HBVAYWE   -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G-----------T--G--T-----A-ATGT---C-----------A--G--T--G--T-
HBVDNA    -------T--A--------CC-A--------C--A-AA---G----G-----A-----A-----A--T-----G--A---------C----G-----------T--G--T-----A-AGGTT--C--------A--A--G--T--G--T-
HPBHBVAA  -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G-----------T--G--T-----A-AGGTT--C-----------A--G--T--G--T-
XXHEPAV   -------T--A--------CC-A--------C--A-AA---G----------A-----A-----A--C-----G--A------C--C----G--------A--T--G--T-----A-ATGTT--C-----------A--G--T--G--T-
HBVORFS   -------T--A---C----CC-A--------C--A-AA---G----------A-----A-----A--------G--AC-----C--C----------------T--G--T-----A-ATGTT--------------A-----T--G--T-
HHVBE4    -----G-T--------G--CC-------------C-AA---G----------A--T-----A--A-----------AC-------A--C---G-C--------T-----T-T---C-T-TCT--A------------C----G-----T-
HHVBBAS   -----G-T--------G--CC-------------C-AA---G----------A--T-----A--------------AC-------A--C---G-C--------T-----T-T---C-T-TCT--G------------C----G-----T-
HHVBF     -----G-T-----------TC----T--G--ATC--AA---G----------A-----G------AA-------TT---------A-----GG-C-----A--T-----T-------AG--T--C--------C-TA--G--G-----G-
HHVBFFOU  -----G-T--------G--TC-A---C-G--ATC--AA---G----------A-----A--T--CAA-------TT------G--------G--------A----------T-----AA-TT-----------C--TC-G--G----AC-
HBVADW4A  -----G-T--------G--TC-A---C-G---TC--AA---G----------A-----A--T---AA-------TTAC-------A-----GG-------A--T-----T-------AGGTT--C--------T---A-G--T-----C-
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                                                                                                                                                       HBPR 1
           2701
HBVXCPS    TTAAACCTTATTATCCAGATCAGGTAGTTAATCATTACTTCCAAACCAGACATTATTTACATACTCTTTGGAAGGCTGGTATTCTATATAAGAGGGAAACCACACGTAGCGCATCATTTTGCGGGTCACCATATTCTTGGGAACAAGAGC
HBVADW     ------------------------------------------------------------------------------------------------------------------------------------------------------
HVHEPB     ----------------------------------------------------------------------------G-----------------A-------------------------------------------------------
S50225     ----------------------------------------------------C---------------------------------------------C---------------------C-C---------------------------
HPBADWZCG  -----A----------T-----T-----------------T------C-------------------------------G--------------------T-----------C-------------------------------------
HPBADW3    -------------------G--T--------------------G--G-----------------------------G-----CT-------A--A--G--A-----------C-------------------------------------
HPBADWZ    -C-----------------G--T--------------------G--G-----------------------------G-----CT-------A--A--G--A-----------C-----------------------------------T-
HPBADW1    -C-----------------G--T--------------------G--T-----------G-----------------G-----CT-------A--A--GT-A----A------C-----------------T-----------------T-
HPBADW2    -C-----G-----------GT-T--------------------G--GC-------------C--------------G--G--CT-------A--A--GT-------------C-----------------------------------T-
HPBCGADR   ----------------T--A--T-C----------------A----T--G-----------------G--------G--C--------------A-----T-----C-----C--------T----------------------------
HBVADRM    ----------------T--A--T-C----------------A----T--G-----------------G--------G--C--------------A-----T-----C-----C--------T----------------------------
HPBADRA    ----------------T--A--T-C----------------A----T--G-----------------G-----------C-----------A--A-----T-----C-----T--------T----------------------------
HPBCG      ----------------T--A--T-C----------------A----T--G--------------G--G-----------C-----------A--A-----T-----C-----T--------T----------------------------
HEHBVAYR   -------G--------T--AT-T-C----------------A----T--G-----------------G-----------C--------------A-----T-----C-----C--------T----------------------------
HBVADR     -------G--------T--A--T-C----------------A----T--G-----------------G-----------C--------------A-----T-----C-----C--------T----------------------------
HBVADR4    -------A--------T--A--T-C----------------A----T--G-----------------G--------G--C--------------A-----T-----C--T--C-----C--T----------------------------
HPBADR1C   -------A--------T--A--T-C----------------A----T--G-----------------G--------G--C--------------A-----T--G--C-----C--------T----------------------------
HPBADRC    ----------------T--A--T-C------------T---A----T--G-----------------G-----------C--------------A-----T--------T--C--------T----------------------------
HBVPREX    ----------------T--A--T-C---------------------T--G-----------------G-----------C--------------A-----T-----C--T--C--------T----------------------------
HPBETNC    ----------------T--A--T-C---------------T-----T--G-----------------G-----------C--------------A-----T--C--C-----T--------T----------------------------
HHVBC      -------------------A---AC----------------A----T--G--------G--------G-------------G------------A-----T-----C-----C--------T----------------------------
HHVCCHA    ----------------T--A--T-C----------------A----A--G-----------------G-----------C--CT-------A--A-----T-----C--T--C--------T----------------------------
HBVAYWMCG  -------G-----------A--TC------------------------------------C-----A--------G--G--AT-----------A-----A----A------C--------T--A------------------------A
HBVAYWC    -------------------A--TC----------------------T-----C--------C-----A--------G-----AT-----------A-----A----A------C--------T-------------------------T-
HBVAYWCI   -------------------A--TC----------------------T-----C--------C-----A--------G-----AT-----------A-----A----A------C--------T-------------------------T-
HBVAYWE    -------------------G--TC-------------------------------------C-----A--------G-----AT-----------A-----A----A------C--------T----------------------------
HBVDNA     -------------------A--TC----------------------T-----C--------C-----A--------G-----AT-----------A-----A----A------C--------T-------------------------T-
HPBHBVAA   -------------------A--TC-------------------------------------C-----A--------G--G--AT-----------A-----T----A------C--------T--------------C------------
XXHEPAV    -------------------AT-TT----------------------T--------------C-----A--------G-----AT----C------A-----T----A------C--------T----------------------------
HBVORFS    ----------------G--A--TT-------------------------------------C-----A--------G-----AT------------------A-----------C--------T----------------------------
HHVBE4     -A------------------A-T-----------------------------C-----------C--A-----------GCA---------A--A-----T-----------C--------T----------------------------
HHVBBAS    -A------------------A-T-----------------------------C-----------C--A--------G--C--CT-------A--A-----T-----------C--------T--------T-------------------
HHVBF      -------C----------------C------------T--T-----------------G------T-A--------G--A--------------A-----------------C--------T-------A------C--------------
HHVBFFOU   -------------------G--T-C------------T--T-----------------G------T-A--------G--A---T----------A---T-------------C--------T----------------------------
HBVADW4A   -C-----C--------T--G--T-C---------------TA----------------G------T-A--------G--A---T----------A---T-------------C--------T--------------C--------------
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                   Start HBpreS1Ag
                   HBPr 2                                                                                                                                                LiPA
                                                                                                                                                                         Probe
HBVXCPS     TACAGC ATGGGAGGTTGGTCATCAAAACCTCGCAAAGGCATGGGGACGAATCTTTCTGTTCCCAACCCTCTGGGATTCTTTCCCGATCATCAGTTGGACCCTGCATTCGGAGCCAACTCAAACAATCCAGATTGGGACTTCAACCCCATC  50
HBVADW      ------ --//////////////////////------------------------------------------------C------------------------------------------CA---------------------------
HVHEPB      ------ -------------------------------------------------------------------------------------------------T----------------------------------------------  22
S50225      ------ ---------G--A-----------------------------------------------------------------------------------------------------------------------------------
HPBADW2CG   ----T- --------------------------------------------C-----------------------------------------------------------------T---------------------------------
HPBADW3     ------ --------------C--C--------AC-------------A--------C--C-----T-----------------------C------------------AA---------CG-------C---------C-------ACA-  57
HPBADW2     ------ --------------C--C--------AC-------------A--------C--C-----T-----------------------C------------------AA---------CG-------C---------C-------ACA-
HPBADW1     ------ --------------T--C--------A--------------A-----------C-----T--C-----------C---------------------------AA----------G-A---------------C-------ACA-
HPBADW2     ------ --------------T--C--------A--------------A-----------C-----T--------------C---------------------------AA----------G-A---------------C-------G-A-
HPBCGADR    ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HBVADRM     ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-  55
HPBADRA     ---G-- --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HPBCG       ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HEHBVAYR    ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HBVADR      ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HBVADR4     ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HPBADR1C    ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G---------------C-------C-------------------A-
HPBADRC     ------ --------------T--C--------AC-------------------------------T-----------------------C----------------------------------------------------------A-
HBVPREX     ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HPBETNC     ------ --------------T--C--------AC-------------------------------T-----------------------C--------------G-------------------------------------------A-
HHVBC       ------ --------------T-----------G-----------------------G--A-----T-----------------------C--------------G----------------------C--------------------A-
HHVCCHA     ------ --------------T--C--------G--------------------------------T-----------------------C----------------------------------------------------------A-
HBVAYWMCG   ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-  92
HBVAYWC     ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-
HBVAYWCI    ------ -----///////////////////////////////////CA-----A---CACCAG--T--------------------C--C--------T--A--C-C-A----A---A-CGCA--------------------T----A-
HBVAYWE     ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-
HBVDNA      ------ -----///////////////////////////////////CA-----A---CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-
HPBHBVAA    ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-TGCA--------------------T----A-  73
XXHEPAV     ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-
HBVORFS     ------ -----///////////////////////////////////CA---------CACCAG--T--------------------C--C--------T--A--C---A----A---A-CGCA--------------------T----A-
HHVBE4      ----T- -----GCT--CT-GGA-GGTC----T-G-///TG------A----A-----CACCA---T-----------T--------C--C--------T--A------A----A---A-C-GA---------------CA---T----A-  60
HHVBBAS     ----T- -----GCT--CT-GGA-GGTC----T-G-///TG------A----A-----CACCA---T-----------T--------C--C--------T--A------A----A---A-C-GA---------------CA---T----A-  61
HHVBF       ------ -------CACCTCTC----CGA-AA-A-GG--------ACA------------G-----T--A-----C-----G--A--C------C----T-GCT-----A----A--T--C--G-G---C--C------------A-A-A-  64
HHVBFFOU    ------ -------CACCTCTC----CGA-AA-A-GG--------ACA------C-----G-----T--A--------------A--C-----AC----T-CTT-----A----A--T--C--G-G---C---------------AAA-A-
HBVADW4A    ------ -------CACCTCTC----CGA-A--A-G---------ACA------C-----G-----T-----------------A--C------C----T-GCT-----A----A--T--C--G-G---C--C------------A-A-A-  63
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             3001                                                                                                                                                    LiPA
                                                                                                                                                                     Probe
HBVXCPS      AATGGACCACTGGCCAGCAGCCAACCAGGTAGGAGTGGGAGCATTCGGGCCAAGGCTCACCCCTCCACACGGCGGTATTTTGGGGTGGAGCCCTCAGGCTCAGGGCATATTGACCACAGTGTCAACAATTCCTCCTCCTGCCTCCACCAAT   15
HBVADW       ----------------------------------------------------G--------------------------------------------------------------------------------------------------------  33
HVHEPB       ----------------------------------------------------G--T------------------G-------------------G------C----------------------------G-----
S50225       -----------------------------G----------------------G-----------------------------------------A--------------------------------------------
HPBADWZCG    ---------------CA-----------------------------T-----G--T----T--C--------A---G------------------------G----C---C--G-G--G--------------------------
HPBADW3      ------A-------G-ACT-----A----G------------------GG-AT----T--A--C--T--G--AC-G------------A--------C-C--A--T---C----GC-----------------------   58, 59
HPBADWZ      ------A-------G-ACT-----A----G------------------GG-AT----T--A--C--T--G--AC-G------------A--------C-C--A--T---C----GC-----------------------
HPBADW1      ------A-------G-AC---C--A----G-------------------G-AT---------C--T--G--AC-G-------------------------C-C--AT-T---C--G--GC-----------------------
HPBADW2      ------A-------G-AC------A----G-------------------G--T---------C--T--G--AC-G------------------C--C-C--A--T---C--G--GC-----------------------
HPBCGADR     -----T-GT------AG--A--T---------C----------------G--TA-C----A-----------C------------------------------A--C----C--G--GCA-----------------------  55
HBVADRM      -----T-GT------AG--A--T---------C----------------G--TA-C----A-----------C------------------------------A--C----C--G--GCA-----------------------
HPBADRA      -----T---------AG--A-T-A--------C-----AC---------G--T-------A-----------C------------------------------A------C--G--GCG---------TT-------
HPBCG        -----T---------AC-GA-T-A--------C-----AC---------G--T-------A-----------C------------------------C-----A------C--G--GC----------------------
HEHBVAYR     -----T---------AG--A--T---------C---------T--T---G--T-------A--------A--CC-----------------------------A---C--C--G--GCA-----------------------
HBVADR       -----T--A------AG--A--T---------C----------------G--T-------A-----------C------------------------------A------C--G--GCA-----------------------
HBVADR4      -----T--T------AG--A--T---------C----------------G--T-------A-----------C-----------G-----------G------A--C----C--GT-GCA-----------------------
HPBADR1C     -----T---------AG--A--T---------C----------------G--T-------A-----------C------------------------------A------C--GT-GCA-----------------------
HPBADRC      -----T--T------AG--A--T---------C---------T------G--T----T--A-----------C------------------------------A------C--G--GCG-------------T------
HBVPREX      -----T---------AG--A--G---------C----------------G--T-------A----------CC-----------------------C------A------C--G--GCG-----------T---------
HPBETNC      -----T--T------AG-----T--------------------------G--T-------A-----------C------------------------------A------C--G--GCG-------------T------
HHVBC        -----T---------AG--A--T---------C----------------G--T-------A--------A--C-----------------C------------A------C--G--GC-------T-------------T------
HHVCCHA      -----T--A------AG--A--T---------C----------------G--T-------A--------A--C-----------------A--A---------A---C--C--G--GC-----------------------
HBVAYWMCG    ------AC-------AC------A--------CT-----------TGG-A-------A--G-----A--CC-----------------------------C-ACAG--C---C--G---A---G------------T------
HBVAYWC      ------AC-------AC------A--------CT-----------TGG-TT------A--G-----A--CC-----------------------------C-ACAA--TT--C--G---A---G-----------------
HBVAYWCI     ------AC-------AC------A---AT---CT-----------TGG-A-------A--G-----A--CC-----------------------------C-ACA---C---C--G---A---G------------T------
HBVAYWE      ------AC-------AC------A--------CT-----------TGG-TT------A--G-----A---C-----------------------------C-ACAT--C---C--G---A---G------------T------   56
HBVDNA       ------AC-------AC------A--------CT-----------TGG-TT------A--G-----A--CC-----------------------------C-ACAA--TT--C--G---A---G-----------------
HPBHBVAA     ------TC-------AC------A--------CT-----------TGG-AT------A--G-----A--CC-----------------------------C-ACAA--CT--C--G---A---G-----------------
XXHEPAV      ------AC-------AC------A--------CT-----------TGG-AT------A--------A--CC-----------------------------C-AGAA--GT--C--G---A---G------------T------
HBVORFS      ------AC-------AC------A--------CT-----------TGG-AT------A--G-----A--CC-----------------------------A-ACAA--CT--C--G---A---G-----------A--T------
HHVBE4       --A--------A-G-A-------A---------------C--------GG--T----T--C--------A--CC-------------------A-----GC-A-AA---T--C--G--GAC--G-----------------
HHVBBAS      --A--------A---A-------A------------------------TG--T----T--C--------A--CC-------------------A-----GC-A-AA---T--C--G--GA---G-----------------
HHVBF        ------AGT------ATG--A--A--------------GC-A---T---G--T----A--C--------T--CC-GC----------------A-----TG-T--A--A--CT--C--G--GA---G-----------T------
HHVBFFOU     ------ACT------ATG--A--A--------------GT-A---T---G--T----A--C--------T--CC-G-----------------A--A--TG-TC-A--A--CT--C--G--GA---G-----------------   65
HBVADW4A     ------AGT------ATG--A--A--------------GC-A---T---G--T----A--C--------T--CC-GC--------------A--A--A--TG-G--A--A--CT--C--G--GA---G-----------T------
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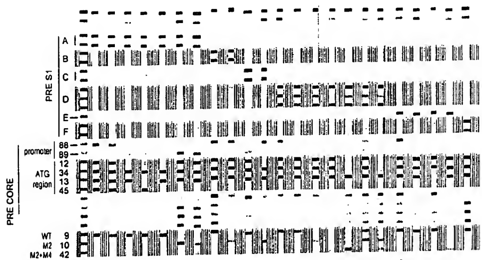
                                                                        HBPr 3       Start HBpreS2Ag
                                                                                                   LiPA
HBVXCPS    CGGCAGTCAGGAAGGCAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGT CATCCTCAGGCC ATGCAGTGG  Probe
HBVADW     --------------------------------------------------- ------------ ---------
HVHEPB     --------------------------------------------------- ------------ ---------
S50225     -----------------------------------------G-----A--- ------------ ---------
HPBADWZCG  --------------------------------------------------- ------------ ---------
HPBADW3    -------T-------A-----------C-G-------------------C- -----------A ---------
HPBADWZ    -------T-------A-----------C-G-------------------C- ------------ ---------
HPBADW1    ------------C--------------C-A--------------G----C- ------------ ---------
HPBADW2    ---------------------------T-A--C-----------G----C- ------------ ---------
HPBCGADR   --------------A------------------------------------ ------------ ---------
HBVADRM    --------------A------------------------------------ ------------ ---------
HPBADRA    --------------A------------------------------------ ------------ ---------
HPBCG      --------------A------------------------------------ ------------ ---------  54
HEHBVAYR   --------------A------------------------------------ ------------ ---------
HBVADR     --------------A------------------------------------ ------------ ---------
HBVADR4    --------------A------------------------------------ ------------ ---------
HPBADR1C   --------------A------------------------------------ ------------ ---------
HPBADRC    --------------A------------------------------------ ------------ ---------
HBVPREX    --------------A------------------------------------ ------------ ---------
HPBETNC    --------------A-----A------------------------------ ------------ ---------
HHVBC      --------------A--------G--------------------------- ------------ ---------
HHVCCHA    --------------A--------G--------------------------- ------------ ---------
HBVAYWMCG  --C---A----------------C--TC-G---------T-G-------C- ------------ ---------
HBVAYWC    --C--------------------C--TC-G---------T-G---A---C- ------------ ---------
HBVAYWCI   --C---A----------A-----C--TC-G---------T-G-------C- ------------ G--------
HBVAYWE    --C--------------------C--TC-G---------T-G---A---C- ------------ ---------
HBVDNA     --C--------------------C--TC-G---------T-G---A---C- ------------ ---------
HPBHBVAA   --C--------------A-----C--TC-G---------T-G---A---C- ------------ ---------
XXHEPAV    --C--------------------C--GC-G---------T-G---A---C- ------------ ---------
HBVORFS    --C--------------------C--GC-G---------T-G---A---C- ------------ ---------
HHVBE4     -----------------------C--A---A--------T-G-------C- ------------ ---------  62
HHVBBAS    -----------------------C--A---A--------T-G-------C- ------------ ---------
HHVBF      ----T---C--G---A----A--C-AAG---------------------CA ------------ ---------
HHVBFFOU   ----T---C--G---A----A--C--AG---------------------CA -----A-----A ---------
HBVADW4A   ----G---C--G--AA----A--C--AG---------------------CA -----A-----A ---------
```

Figure 2: LiPA HBV design				
			HBPr	
LiPA line	Region	Purpose	Probe number/SEQ ID NO	sequence
0		Pencil line		
1		biotinylated DNA		
2	PreS1	ampl. contr.	33	CTGAGGGCTCCACCCCA
3	PreS1	Genotype A	22	AACCTCGCAAAGGCAT
4	PreS1	Genotype A	50	CCCAGAGGGTTGGGAAC
	PreS1	Genotype A	15	GCCAGCAGCCAACCAG
5	PreS1	Genotype B	57	CTGCATTCAAAGCCAACT
	PreS1	Genotype B	58	CCCCATGGGGGACTGTTG
6	PreS1	Genotype B	59	CATACTCACAACTGTGCCA
7	PreS1	Genotype C	55	TTCAACCCCAACAAGGATC
8	PreS1	Genotype C	54	TCAGGAAGACAGCCTAC
9	PreS1	Genotype D	92	TTCTGCCCCATGCTGTA
10	PreS1	Genotype D	56	AATGCTCCAGCTCCTAC
11	PreS1	Genotype D	73	TTCCACCAGCAATCCTC
12	PreS1	Genotype E	60	GGGCTTTCTTGGACGGTCC
	PreS1	Genotype E	61	CTCTCGAATGGGGGAAGA
	PreS1	Genotype E	62	CCTACCCCAATCACTCCA
13	PreS1	Genotype F	63	AGCACCTCTCTCAACGACA
14	PreS1	Genotype F	64	GCAAATTCCAGCAGTCCCG
	PreS1	Genotype F	65	GCCAATGGCAAACAAGGTA
15	preCore	promotor	88	TAGGTTAAAGGTCTTTGT
16	preCore	promotor	89	TAGGTTAATGATCTTTGT
17	preCore	scan codon -2 to +3	12	AAGTTGCATGGTGCTG
18	preCore	scan codon 1 to 5	34	ATGCAACTTTTTCACC
19	preCore	scan codon 5 to 9	13	CACCTCTGCCTAATCAT
20	preCore	scan codon 12 to 17	45	TGTACATGTCCCACTGTT
21	preCore	scan codon 12 to 17	46	TGTTCATGTCCTACTGTT
22	preCore	scan codon 16 to 20	47	ACTGTTCAAGCCTCCAAG
23	preCore	scan codon 19 to 23	48	GGCACAGCTTGGAGGCTT
24	preCore	scan codon 23 to 27	49	AAAGCCACCCAAGGCACA
25	preCore	codon 28 wt	9	TGGCTTTGGGGCATGG
26	preCore	codon 28 mt	10	TGGCTTTAGGGCATGG
27	preCore	codon 28+29 mt	42	TGGCTTTAGGACATGGA



Genotype	A	A	A	A	A	A	A	B	B	C	C	D	D	D	D	D	D	D	E	E	E	E	F
precore promoter	W	mix?	M?	M?	I	M	M	W	W	M	W	W	I	I	I	I	W?	I	W	I	I	I	I
ATG-region	W	W	M	M	M	W	W	W	W	W	M	W	W	W	W	M	W	M	W	W	M	W	W
codon15	W	W	M	W	M	M	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
codon 28	W	W	W	W	W	M	M	W	mix	W	M	W	W	M	M	M	mix	mix	W	mix	W	M	W
WT									+								+			+			
M2						+	+		+		+			+			+	+					
M2+M4							+								+	+		+		+		+	
HBeAg	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-	
α-HBe	-	+	+	+	+	-	+	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	

I = indeterminate, W = wild type, M = mutant

6

Abstract

The present invention relates in a first aspect to a method for determining the presence or absence of one or more HBV genotypes in a biological sample, comprising hybridizing the polynucleic acids of the sample with at least two nucleotide probes hybridizing specifically to a HBV genotype specific target sequence chosen from Figure 1, with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of the sample under the same hybridization and wash conditions; and detecting the hybrids formed; and inferring the HBV genotype present in said sample from the differential hybridization signal(s) obtained.

The invention also relates to a similar method for determining the presence or absence of one or more HBV mutants in a biological sample, comprising hybridizing with at least two nucleotide probes hybridizing specifically to a HBV mutant target sequence chosen from Figure 1.

Preferably, the present invention relates to a method for HBV genotyping and determination of the presence or absence of one or more HBV mutants in one single experiment.

The invention further relates to sets of nucleotide probes and possibly primers useful in said methods as well as to their use in a method for typing and/or detecting HBV and to assay kits using the same.